Synthesis of novel heterocyclic constraints as probes for peptide bioactive conformation

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SYNTHESIS OF NOVEL HETEROCYCLIC CONSTRAINTS AS PROBES FOR PEPTIDE BIOACTIVE CONFORMATION

Submitted by Lai Chun Chan

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To my Mother and Father

ABSTRACT

This thesis describes our own approach for the construction of turn mimetics (γ- and inverse γ-turn) and for an α-helix constraint, based on the heterocyclic skeleton of 4-amino-3-isoxazolidinone.

A review of the types of secondary structures and turns found in peptides, and their putative importance for bioactivity is described. This is followed by an account of the different types of peptide mimetics that have been synthesised and incorporated into bioactive peptides for structural-activity studies.

A detailed study of the imidoyl-X function in the dihydroisoxazole ring (where X = Cl, Br, OSO_2Me and OSO_2CF_3) as a possible route to incorporate amino esters is reported. The generation and the incorporation of the 3-chloro-4-amino-4,5-dihydroisoxazole as a potential heterocyclic constrained glycine mimetic, into the C-terminus of a peptide chain has been achieved. Conformational studies by NMR have been applied to examine the "constrained" peptide for a γ- and inverse γ-turn feature.

Work applied to the synthesis of the more constrained variants; cis-fused bicycle **II** (as an inverse γ-turn mimetic), bridged bicycle **III** (as a new type of constraint) and spiro-fused bicycle **IV** (as an α-helix constraint) is also described.

Finally, the potential use of chloromethoxyphthalimde and N,N′-bis(BOC)aminoxychloromethane as a bifunctional equivalent to $NH_2OCH_2^{\oplus}$ is reported.

CONTENTS

ABBREVIATIONS

The following abbreviations are used in the text:

Ac	acetyl
AIBN	azobisisobutyronitrile
Bz	benzyl
BOC	*tert*-butyloxycarbonyl
br	broad
c	concentration
cat	catalytic
Cbz	benzyloxycarbonyl
C.I.	chemical ionisation
δ	chemical shift
dec.	decompose
DCC	1,3-dicyclohexylcarbodiimide
DDPA	Diphenylphosphoryl azide
DIEA	Diisopropylethylamine
DMAP	4-(dimethylamino)pyridine
DMF	dimethylformamide
DMSO	dimethyl sulphoxide
E.I.	electron impact
eq	equivalent
FAB	fast atom bomdardment
FMOC	9-fluorenylmethyoxycarbonyl
h	hour
HOMO	highest occupied molecular orbital
HOBt	1-hydroxybenzotriazole hydrate
HPLC	high performance liquid chromatography

IR	infra red
J	coupling constant
LDA	lithium diisopropylamine
LUMO	lowest unoccupied molecular orbital
m	meta
M	molar
M^+	molecular ion
min	minutes
m.p.	melting point
Ms	methanesulphonyl
n	straight chain
NBS	N-bromosuccinimide
NMM	N-methylmorpholine
NMR	nuclear magnetic resonance
Nu	nucleophile
o	ortho
p	para
py	pyridine
ppm	parts per million
ROESY	rotating frame overhauser enhancement spectroscopy
RT	room temperature
tert	tertiary
^{t}Bu	*tert*-butyl
THF	tetrahydrofuran
TLC	thin layer chromatography
TMS	trimethysilyl
TROC	2,2,2-trichloroethyoxycarbonyl
Tos	4-methyphenylsulphonyl
Xs	excess

The abbreviated terms for the 20 naturally occurring α-amino acids are taken from the 'Greenstein and Wintz'. [83]

Name	Abbreviation
Alanine	Ala
Arginine	Arg
Aspartic acid	Asp
Cysteine	Cys
Glutamic acid	Glu
Glycine	Gly
Histidine	His
Hydroxylysine	Hylys
Hydroxyproline	Hypro
Isoleucine	Ile
Leucine	Leu
Lysine	Lys
Methionine	Met
Phenylalanine	Phe
Proline	Pro
Serine	Ser
Threonine	Thr
Tryptophan	Try
Tyrosine	Tyr
Valine	Val

INTRODUCTION

INTRODUCTION

1. Importance of Constrained Peptides

Over recent years there has been intense interest in determining the relationship between three dimensional structure (conformation) and biological activity (binding and transduction) in small polypeptide hormones and neurotransmitters. This was sparked by the tremendous potential offered by not only gaining valuable understanding of the bioactive conformation of a peptide at its receptor/acceptor site but also from the use of this information to synthesise more stable and potent analogues as drug candidates. Early work centred on the substitution and deletion of amino acids in the peptide chain in order to determine the minimum active sequence required for bioactivity. This was then followed by systematic substitution of an L-amino acid by the corresponding D-amino acid which provided insight into the stereostructural requirement of a specific residue for peptide-receptor interaction. Side chain residues were similarly probed by replacement with a methyl group or by pseudoisosteric groups possessing different electronic properties. This enabled the examination of the importance of different side chains and their stereoelectronic properties for interaction with the receptor. These studies have also been greatly assisted by the use of rapid semi-automatic solid phase synthesizers, as a means of generating a wide variety of substrates in a straight-forward fashion.

An alternative way of minimising the conformational flexibility of a target peptide is by introduction of a constrained analogue. The premise for this approach was that an appropriate conformational constraint would restrict a residue, or a group of residues, to a small region of conformational space. Thus, when a peptide interacts with its receptor, or an acceptor molecule such as an enzyme, the conformation required for binding will bear a close relationship to that

seen in solution, given that there will be a high free energy barrier preventing alteration of a constrained conformation.[2]

1.1 Examples of Backbone Constraints Commonly Used

There are numerous types of constraints that can be applied to restrict peptide conformational flexibility. The most general approach involving the introduction of a non-covalent modification to the backbone of the polypeptide chain provides insight to conformational stability and the importance of secondary structures in polypeptides and proteins.

The simplest form of a backbone constraint involves the modification of the amide bond itself. This field has been extensively explored and is the subject of a comprehensive review by Spatola.[1] The different types of constraints that are most widely applied to restrict flexibility and hence the conformational space that a peptide can occupy are illustrated below:

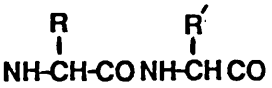
i. Substitution of (D) for the naturally-occurring (L)-amino acids. This common manipulation involves the inversion of stereochemistry of the α-carbon atom in an amino acid component of a peptide and results in the generation of a diastereomeric system.

ii. Methylation of the α-C or of the nitrogen of the peptide bond (CO.NH replaced with CO.NMe). This type of constraint reduces the hydrogen bonding capacity of nitrogen as well as increasing the steric bulk associated with the side chain.

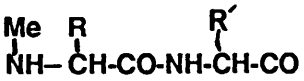
iii. Desipeptide analogues where NH in CO.NH is replaced by O or S to give either an ester (CO.O) or a thioester (CO.S) linkage.

iv. Intercalation of methylene into the peptide backbone using a β-amino acid of which there are two possible arrangements i.e. NH-CHR-CO replaced

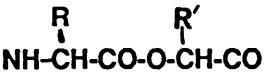
Scheme I



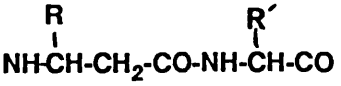
Linear peptide



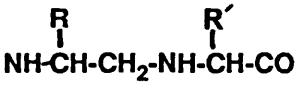
N-methyl

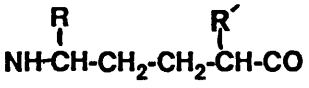


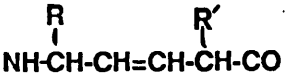
Desipeptide



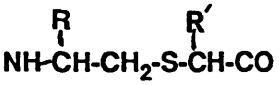
Intercalation



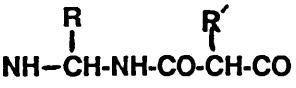




Carba-replacement

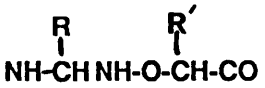


Thiomethylene

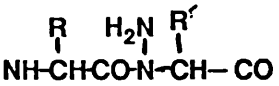


Retro-peptide

Hydrazino-peptide



Aminoxy-peptide



N-amino-peptide

by either NH-CH_2-CHR-CO or NH-CHR-CH_2-CO.

v. Carba replacement involves modification of the amide group itself. For example, CO.NH may be replaced by CH_2CO, CH_2NH, CH_2CH_2 or CH=CH. These modifications will alter the stability and conformational preference of structures containing important hydrogen bonded features.

vi. The use of retro-peptides which involves reversal of the amide bond (-CO.NH- to -NH.CO-).

vii. Other changes can be carried out on the α-amino group. This may involve replacing the functional group by analogues based on hydrazino (NH replaced with NHNH), aminoxy (NH replaced with NHO), and N-amine (NH replaced with NNH_2).

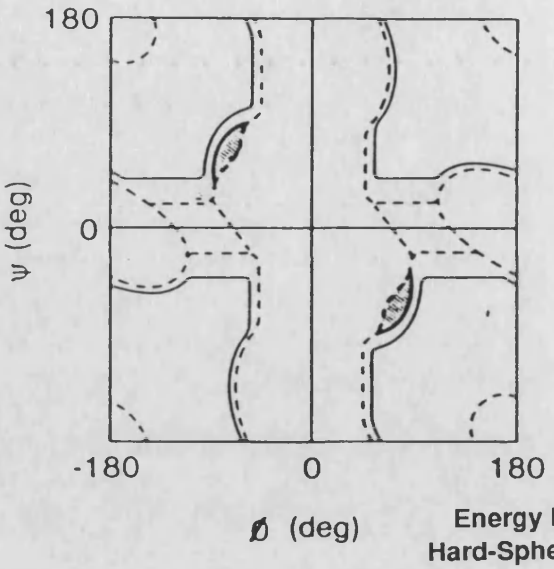
For more details see Spatola[1] and Hruby[2] and Scheme I.

These types of amide bond replacement provide insight into the importance of specific amide bonds and amino acid residues for ligand-receptor (acceptor) interactions. These modifications may also serve to stabilise peptide substrates against enzymatic degradation and are also of use in the design of novel protease inhibitors.

1.2 Allowed and Disallowed Conformational Space

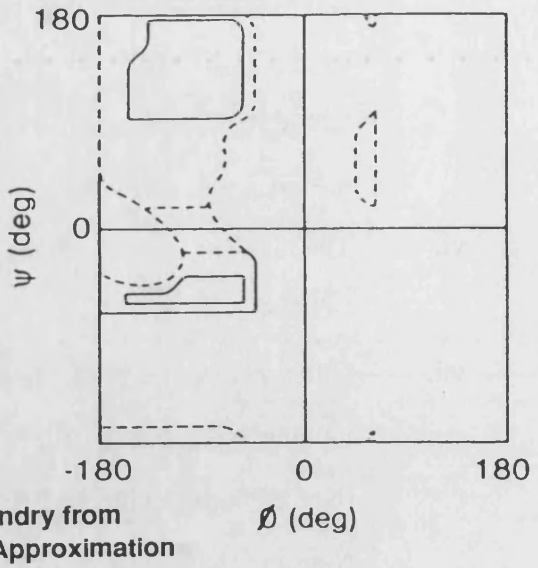
One important way of visualising the influence of constraints on individual amino acids was pioneered by the group of Ramachandran and Sasisekharan.[4] This group used hard-sphere approximations to calculate the non-bonding interaction between atoms as a function of the torsion angles ϕ and ψ. In this method, atoms were assumed to be hard spheres of fixed radii (usually the van der Waals radius) and these spheres were not allowed to overlap. This permitted the conformations of peptides to be categorized into regions of

Fig. 1A



(N-acetyl-Gly-N′-methylamide)

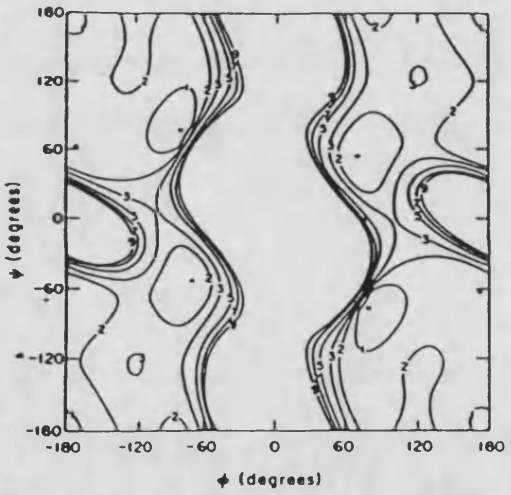
Fig. 1B



(N-acetyl-Ala-N′-methylamide)

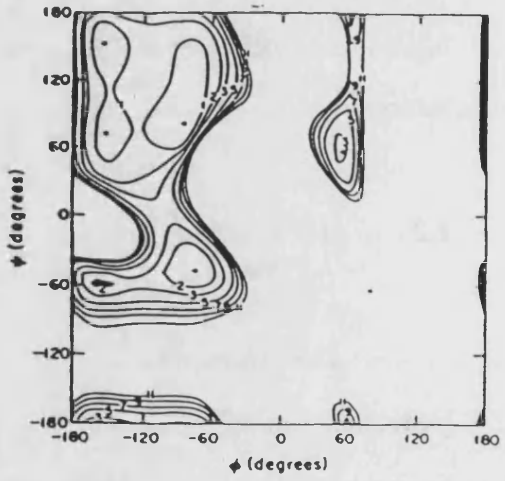
Energy Boundry from
Hard-Sphere Approximation

Fig. 2A



(N-acetyl-Gly-N′-methylamide)

Fig. 2B



(N-acetyl-Ala-N′-methylamide)

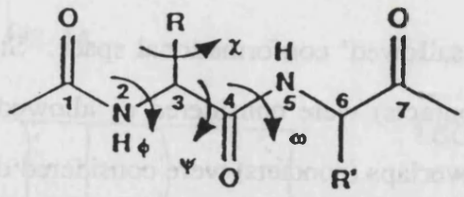
Energy Minima Conformations

energetically 'allowed' and 'disallowed' conformational space. Structures having no hard-sphere overlap (no contacts) were considered as allowed conformations and those having hard-sphere overlaps (contacts) were considered disallowed. The allowed and disallowed conformational space of a particular molecule is expressed in what is known today as the *Ramachandran Map*. This is a two-dimensional graphical representation of a three-dimensional situation, where two variables (ϕ and ψ) are plotted against one another within an 'allowed' energy boundary (Figures 1A and 1B). Figure 1A shows that 50% of the plot of possible conformational space is accessible to glycine (N-acetyl-Gly-N′-methylamide) and that addition of a single methyl group to the α-carbon (to generate alanine) to give N-acetyl-Ala-N′-methylamide decreases this accessible area to 16% (Figure 1B).

The hard-sphere approximation method was later refined to include minimum energy conformational calculations. This approach is more representative of a real system, where spontaneous shifting from a higher energy state to a lower energy state can occur (if a pathway is available). There are various computational programmes available to calculate the energy minima of a peptide.[4b] By fixing both bond lengths and bond angles and by assuming that the peptide bond is in the *s-trans* configuration and with no account being made for rotation of side chain groups, the conformational energy of the peptide is calculated as a function of the torsion angles ϕ and ψ. The conformational properties of the peptide can then be represented as an energy contour map, where torsion angles ϕ and ψ are plotted against one another and the conformational energy levels are represented by contours (Figures 2A and 2B). The local energy minima are usually marked by small dots and these are determined by separate minimization calculations. No contour lines are drawn in the energetically forbidden regions (above 11 kcalmol^{-1}).

The contour maps shown in Figures 2A and 2B have similar features to

Scheme II



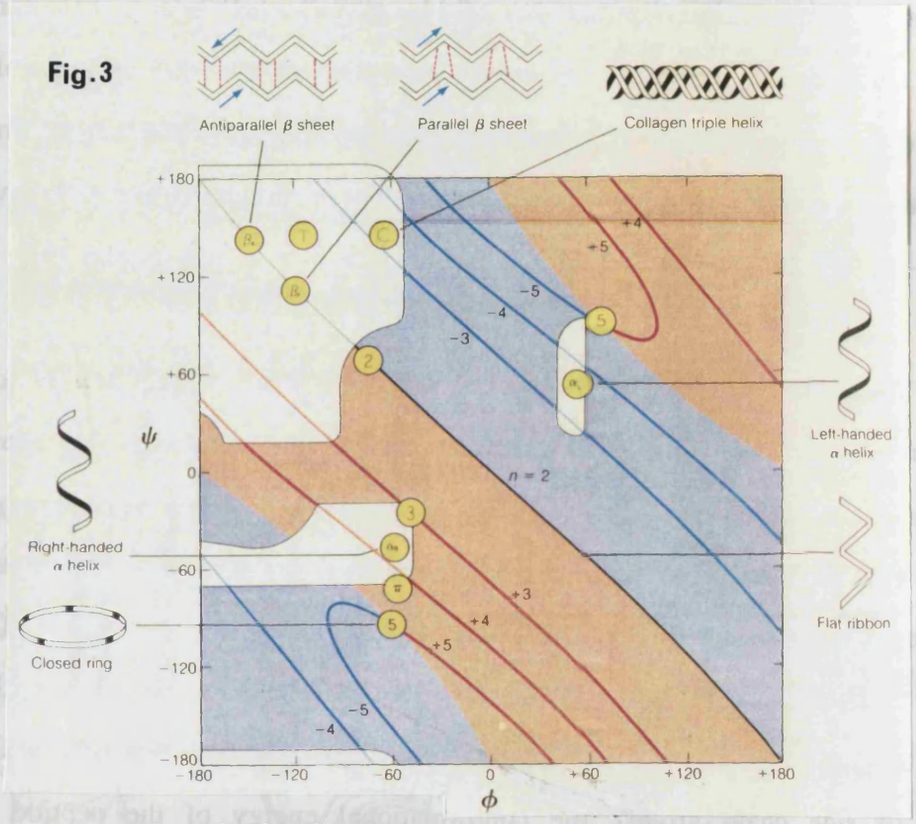
ϕ is the torsion angle between $C_1 N_2 C_3 C_4$

ψ is the torsion angle between $N_2 C_3 C_4 N_5$

ω is the torsion angle between $C_3 C_4 N_5 C_6$ (amide bond)

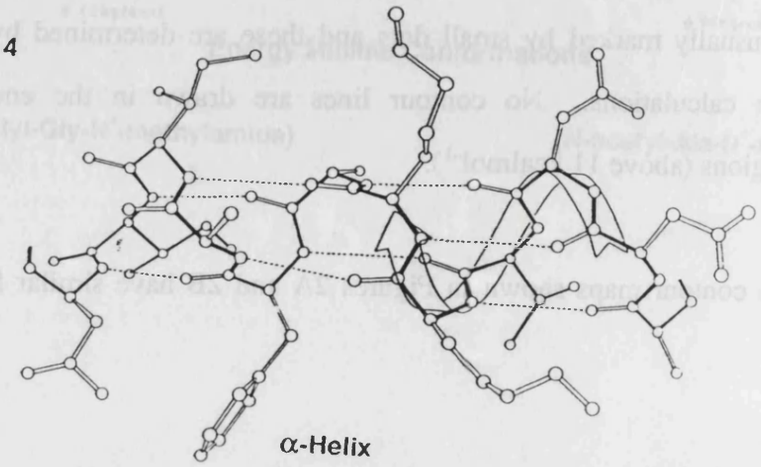
χ is side chain dihedral angle

Fig.3



A Ramachandran diagram.
The white areas correspond to those allowed when side chains are alanine.
Cicles with symbols correspond to important secondary structures:
α_R, α_L = right and left α-helix, β_P, β_A = parallel and antiparallel βsheet
T = twisted β sheet-parallel or antiparellel, 3 = 3_{10}helix, Π = 4.4_{16}helix
5 = five membered ring, 2= twofolded ribbon, C = collagen helix.
The dark contour lines running across the graph correspond to various valves of of n. Where n = positive, the helix is right handed, where negative, it is left handed. Areas for right helices are in red, those for the left helices in blue.

Fig. 4



α-Helix

those obtained by the hard-sphere approximation method (Figures 1A and 1B). The former are, however, more informative by showing that an amino acid residue can exist in several low energy minima states, rather than just one predominant structure.

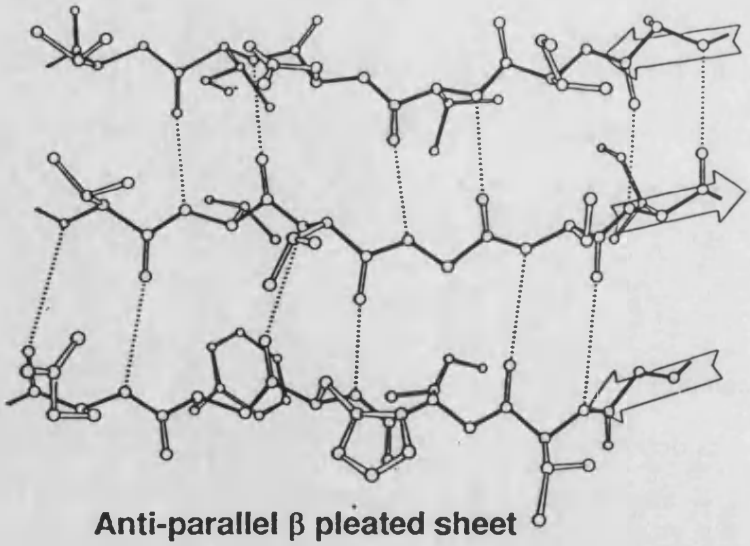
The notation used for torsion angles ϕ and ψ to describe the backbone of a peptide chain was defined by IUPAC-IUB in 1969[5] and is represented in Scheme II.

Even though the hard sphere approximation method may be considered to be primitive and an over simplification, the results are nonetheless instructive, as secondary structural units, such as turns, sheets and helixes, are found to be in regions of allowed conformational space.[4] This indicates that the interresidue hydrogen bonds present in these ordered structures may be established, without having to overcome unfavourable steric contacts between adjacent residues (Figure 3).

1.3 Secondary Structures and Turns in Peptide and Proteins

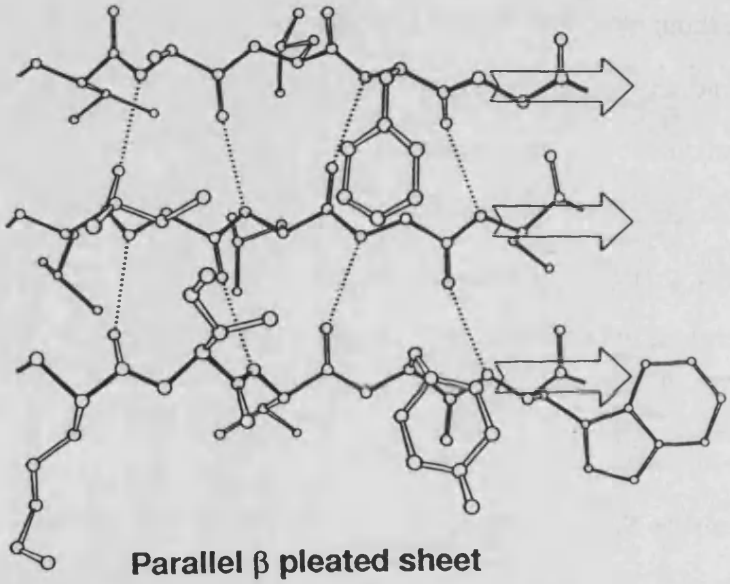
One should be aware of the different types of secondary structures and turns that a peptide chain can exhibit.[6] The most commonly occurring secondary structure found in polypeptides and proteins is the α-helix. An α-helix is used to describe a single strand of peptide chain, twisted in either a right-hand (RH) or a left-hand (LH) direction to form a helical structure, with 3.6 amino acid residues per turn and a hydrogen bond between the C=O of residue n with the NH of residue n+4 (Figure 4). The nomenclature for describing the basic structure of polypeptide α-helix is 3.6_{13}-helix, where 13 is the number of atoms in the hydrogen-bonded loop. The backbone conformation for a RH α-helix may be described by ϕ=-60° and ψ=-60° and for the LH α-helix, ϕ=+60° and ψ=+60°. The 3_{10}-helix can also

Fig. 5



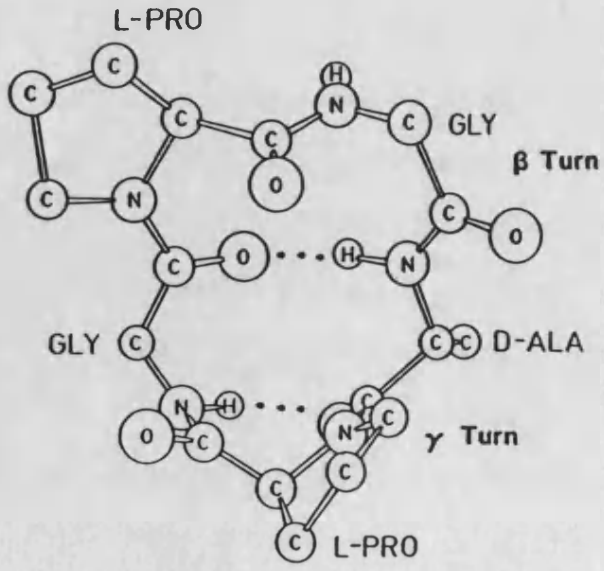
Anti-parallel β pleated sheet

Fig. 6



Parallel β pleated sheet

Fig. 7



Structure of the cyclic pentapeptide cyclo-(Gly–Pro–Gly–D-Ala–Pro) from X-ray diffraction (Karle, 1978)

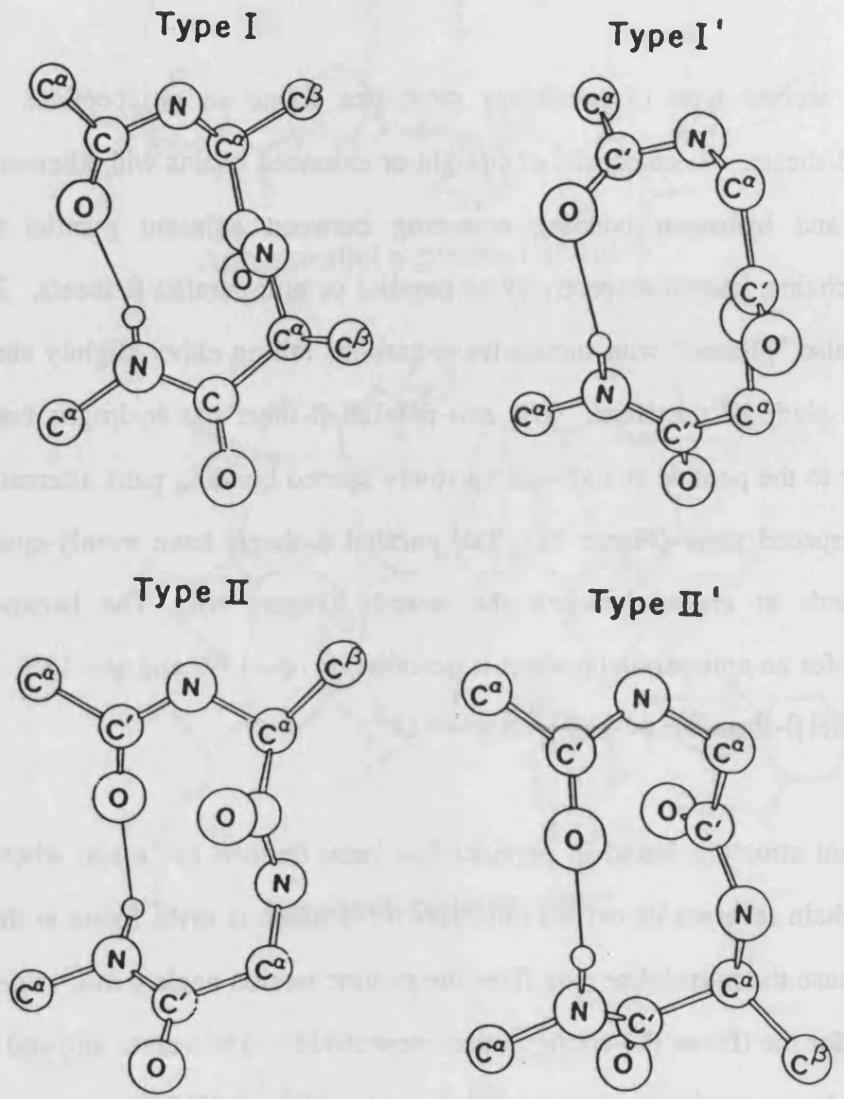
occur and these units are found extensively in globular proteins. This feature has 3 residues repeating and hydrogen bonding occurring at residue n+3 instead of n+4 and has a backbone conformation ϕ=-60° and ψ=30°. This is also a "tighter" helix with only 10 atoms in the hydrogen-bonded loop.

The second type of secondary structures found in polypeptides and proteins are β-sheets. These consist of straight or extended chains with alternating side chains and hydrogen bonding occurring between adjacent parallel and anti-parallel chains, known respectively as parallel or anti-parallel β-sheets. The β-sheets are also "pleated" with successive α-carbons falling either slightly above or below the plane of the sheet. The anti-parallel β-sheet has hydrogen bonds perpendicular to the peptide strand with narrowly spaced bonding pairs alternating with widely spaced pairs (Figure 5). The parallel β-sheets have evenly-spaced hydrogen bonds at angles between the strands (Figure 6). The backbone conformation for an anti-parallel β-sheet is describe by ϕ=-139° and ψ=+135° and that of a parallel β-sheet by ϕ=-119° and ψ=+113°.

A turn structure found in peptides has been defined as "a site where a polypeptide chain reverses its overall direction".[8] Proline is often found at these bends,[8] because the pyrrolidine ring fixes the proline torsion angle ϕ at a value of -60° or +60° for the (D) or (L)-configuration respectively. The values allowed for the corresponding ψ torsion angle are approximately -55° and 130° (D-proline) and 55° and -130° (L-proline). The torsion angles ϕ=-60° and ψ=-55° lead to partial reversal in the direction of the peptide chain and, as a result, proline is often used as a constraint, to induce a "turn" in a polypeptide chain (Figure 7).

There are two main types of turns that we need to consider which are known as β-turns and γ-turns. A β-turn is established when hydrogen bonding occurs between the C=O of residue i with the NH of residue i+3 (via a ten

Fig. 8



β-Turns

membered ring). A γ-turn and its inverse γ-turn occurs when a hydrogen bond is formed between the C=O of residue i with the NH of residue of i+2 (via a seven membered ring) (Figure 9).

There are three main types of β-turns: I, II and III with corresponding mirror images I′, II′ and III′. These structural features were first described by Venkatachalan in 1968[9] from theoretical conformational analysis and the dihedral/ torsion angles (ϕ and ψ) of these types of turns were later determined by Nemethy and Printz (1972)[10] (Table I). A Type III β-turn has repeating values of ϕ and ψ angles of -60° and -30° and is described as a helical turn similar to the 3_{10} helix.[6] Type I and II β-turns are non-helical and the peptide chain is folded back onto itself, so that the first and fourth α-carbons are only 5Å apart (Figure 8). Type I and Type II differ from one another by rotation of the central peptide unit by a 180° flip.

A new category was devised to accommodate the types of turns observed that were of a combination of the above three main types of β-turns.[11,6] A type IV turn is used to describe bends that have at least two of the four torsion angles with values >40°. Type V and V′ turns correspond to torsion angles values of a seven membered hydrogen bonded ring conformation ie. $\gamma(C^{eq}_7)$ or inverse $\gamma(C^{ax}_7)$ turn and is often described as a combination of two γ-turns. A Type VI bend consists of a *cis* peptide link between residues i+1 and a proline residue at i+2 in the polypeptide chain. Finally, the Type VII turn is used to describe a peptide chain where the first and fourth α-carbon are <7Å apart and where a large torsion angle at ψ_{i+1}(~180°) is coupled with a low value of ϕ_{i+2} (<60°) or where a low value of ψ_{i+1}(<60°) is coupled with a large valve of ϕ_{i+2} (~180°) is observed. These often occur as 'kinks' in polypeptide chains and therefore are not true reverse turn structures.

Fig. 9

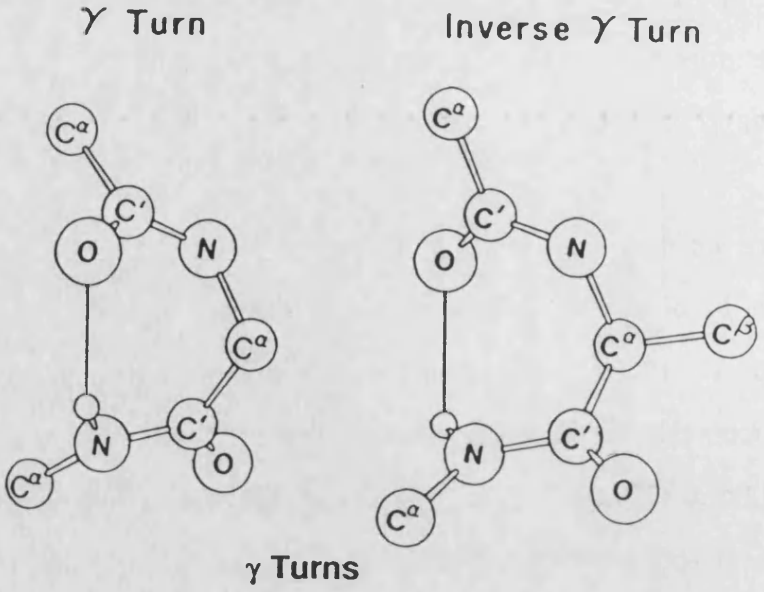


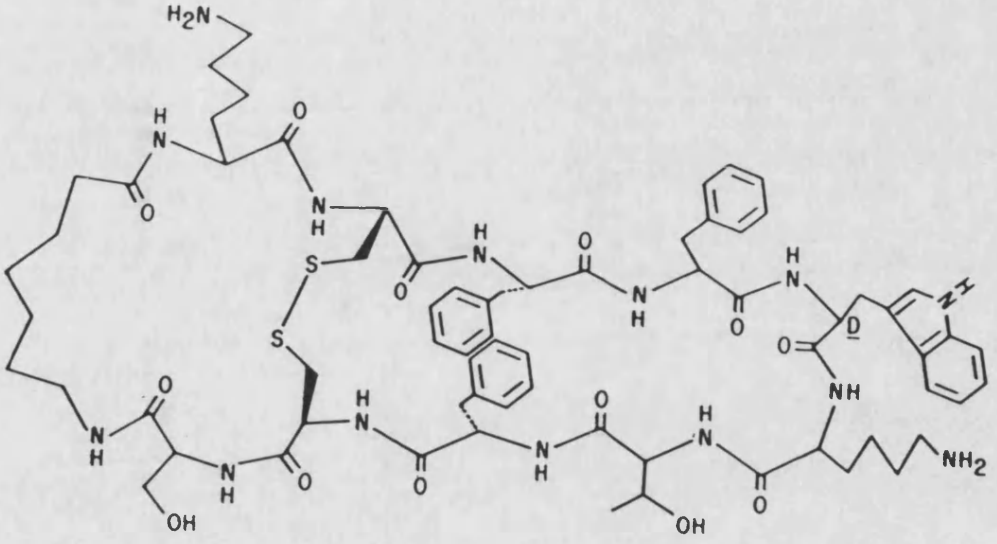
Table I

Types Turns	ϕ_{i+1}	ψ_{i+1}	ϕ_{i+2}	ψ_{i+2}
β turns				
Type I	-60°	-30°	-90°	0
Type I'	+60°	+30°	+90°	0
Type II	-60°	+120°	+80°	0
Type II'	+60°	-120°	-80°	0
Type III	-60°	-30°	-60°	-30°
Type III'	+60°	+30°	+60°	+30°
Type V'	-80°	+80°	+80°	-80°
Type VIa	-60°	+120°	-90°	0
Type VIb	-120°	+120°	-60°	0
γ Turns				
Turn	70-80°	-60 to -70°		
Inverse Turn	-70 to -85°	60-70°		

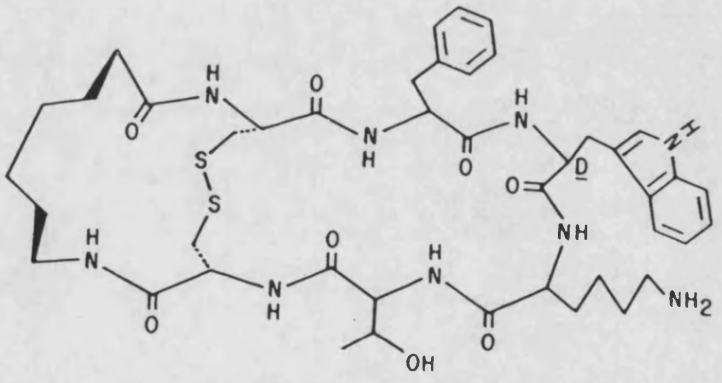
1.4 Cyclic Constraints

The second type of constraints, covalent modifications, involves the formation of cyclic peptides by tying the two end termini together or by cyclising a side chain onto the backbone of the peptide chain. Prior knowledge of the bioactive amino acid sequence is required before this technique can be applied and the formation of cyclic peptides of this type has been reviewed by Hruby *et al.*[2,12] and by Rose *et al.*[8] These cover the methods available for synthesizing shorter cyclic peptides, together with modifications of the backbone of the peptide chain. In addition, with the aid of conformational analysis, the elucidation of the transduction processes and the synthesis of more stable peptide hormone analogues has been possible. One such example is somatostatin, a cyclic tetradecapeptide with the amino acid sequence Ala^{1}-Gly^{2}-Cys^{3}-Lys^{4}-Asn^{5}-Phe^{6}-Phe^{7}-Trp^{8}-Lys^{9}-Thr^{10}-Phe^{11}-Thr^{12}--Ser^{13}-Cys^{14}. This peptide is produced by the hypothalmus and by the endocrine glands and inhibits the release of glycogen, insulin, gastrin, secretin and growth hormone. Interest in somatostatin lies in its potential use to control diabetes. Verber *et al*[10,11,8] have systematically synthesised smaller and less flexible analogues of somatostatin and deduced the amino acid sequence that was essential for bioactivity which was shown to be Phe^{7}-Trp^{8}-Lys^{9}-Thr^{10}. By substituting D-Trp^{8} into this sequence, an increase in somatostatin bioactivity was observed. This, together with the use of NMR and computation conformational analysis, suggested the presence of a Type II′ β-turn in the bioactive conformation of somatostatin. This effort led eventually to the synthesis of a highly potent cyclic hexapeptide analogue of somatostatin (Figure 10).

Fig. 10

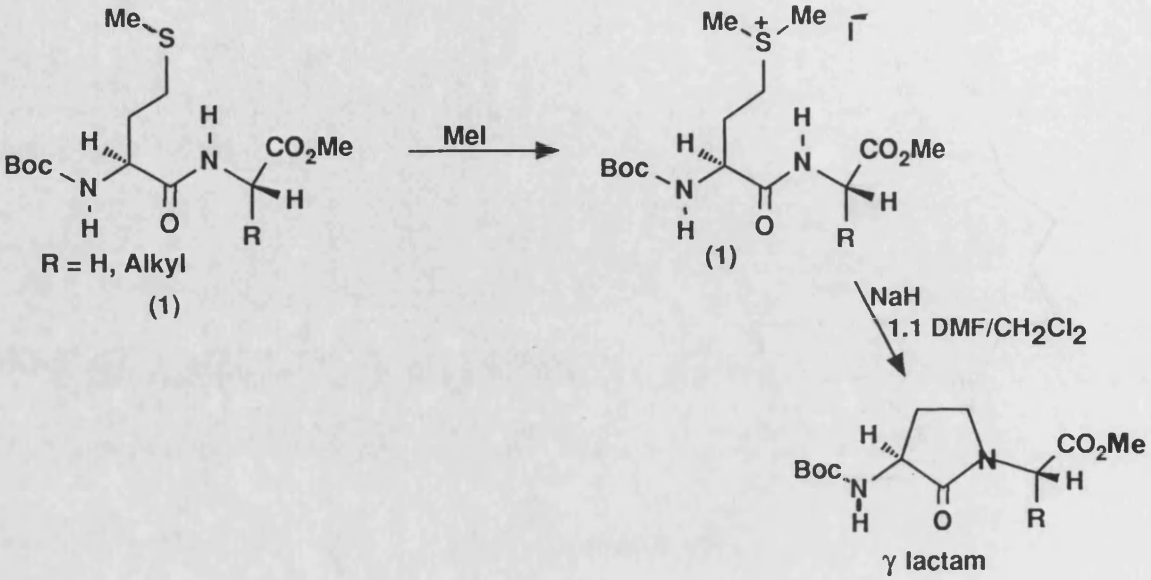


Somatostatin

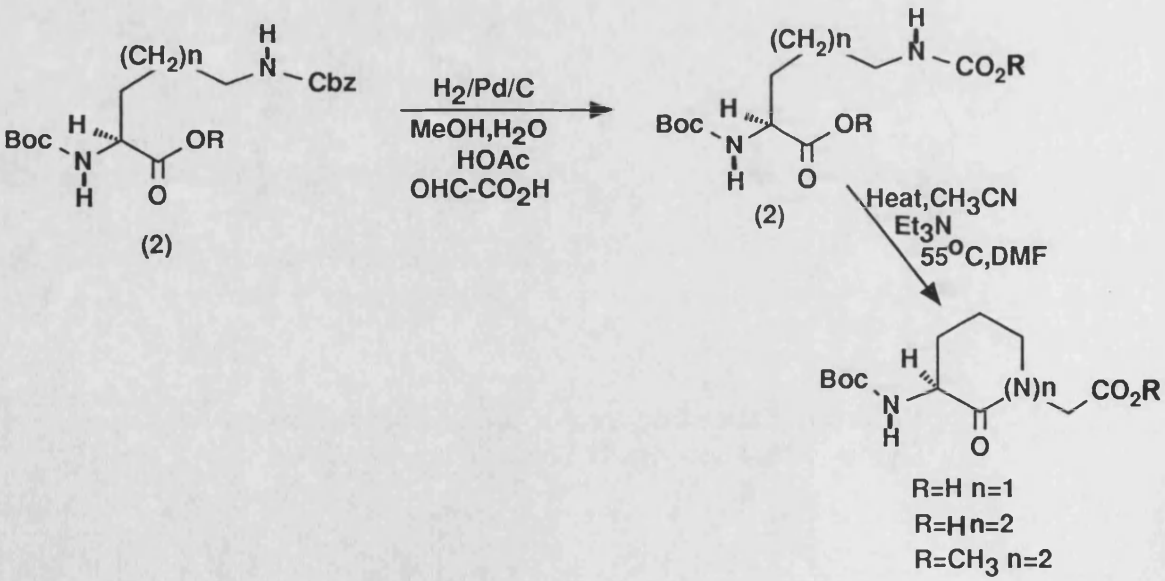


Potent hexapeptide analog of somatostatin
Cyclo-(Aha-Cys-Phe-D-Trp-Lys-Thr-Cys)

Scheme III



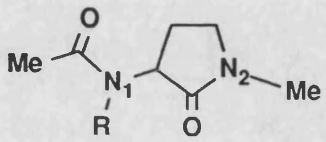
Scheme IV



Cyclisation of side chains to the backbone of the peptide chain has produced a wealth of information regarding the range of turns and secondary structures found in bioactive peptides.[8] The use of γ, δ, ϵ-lactams as a constrained glycine unit with a fixed *trans* amide bond has been described. Freidinger has demonstrated the ease of formation of 5, 6, and 7-membered lactams by intramolecular alkylation of methionine dipeptide sulfonium salt (**1**) (Scheme III) to afford the γ-lactam and by intramolecular cyclisation of the dipeptide ornithine-lysine derivative (**2**) to the δ and ϵ-lactams (Scheme IV).[15] Since then, other routes to lactam constraints of this type have been devised.[16a,b]

The γ and δ-lactams are mainly used to promote a β-turn conformation in a peptide chain, though there have been recent doubts cast over the validity of these constraints.[17a,b,c] Computational studies by Osguthorpe have shown that β-turn conformations are not favoured for a γ-lactam, unless the γ-lactam is appropriately modified to contain a bulky substituent (ie.*tert*-butyl), on the adjacent amide residue 1 (Figure 10b), otherwise an extensive conformation is observed.[17c]

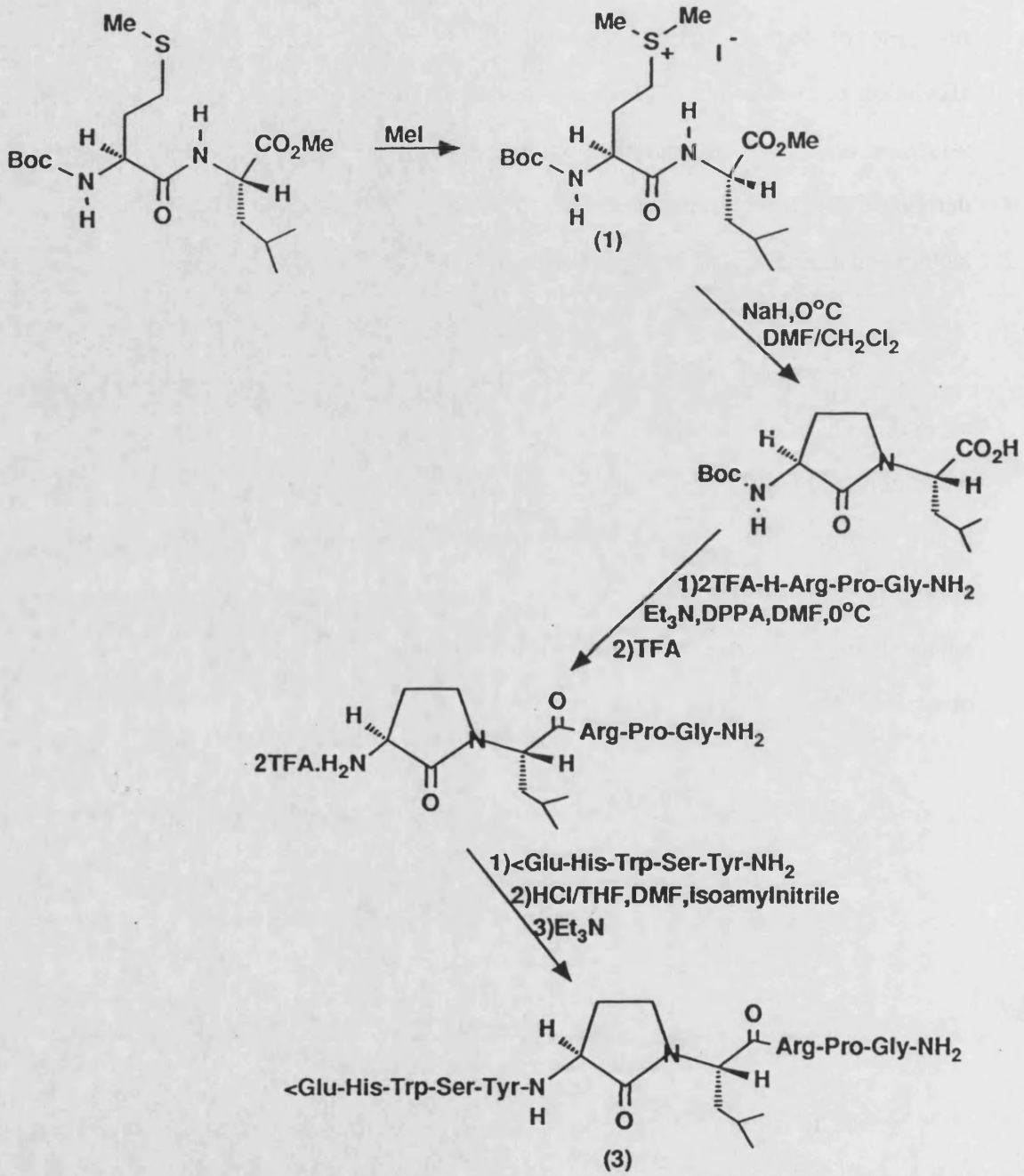
Fig. 10b



R = H, Me, ^{t}Bu

The first reported use of γ-lactam as a conformational constraint was in studies relating to lutenising hormone-releasing hormone (LH-RH)[18] which is produced by the hypothalamus and regulates the levels of pituitary hormones, lutenizing hormone (LH) and follicle-stimulating hormone (FSH). The levels of LH and FSH in turn, regulate the reproductive processes of ovulation and spermatogensis. The potential use of LH-RH and its analogues lies in the

Scheme V


MeI
(1)
NaH,O°C
DMF/CH2Cl2
1)2TFA-H-Arg-Pro-Gly-NH2
Et3N,DPPA,DMF,0°C
2)TFA
Arg-Pro-Gly-NH2
1)<Glu-His-Trp-Ser-Tyr-NH2
2)HCl/THF,DMF,Isoamylnitrile
3)Et3N
(3)

treatment of infertility and as contraceptive agents.[19] LH-RH is a decapeptide with amino acid sequence Glu^1-His^2-Trp^3-Ser^4-Tyr^5-Gly^6-Leu^7-Arg^8-Pro^9--Gly^{10}-NH_2. By examining systematic modification of each residue for biological activity, it was found that replacement of Gly^6-Leu^7 for the constrained variant D-Ala^6-N-Methyl-Leu^7 led to an increase in potency. From conformational energy calculations, this result was shown to be consistent with a β-turn as a biologically important conformation. To investigate the bioactive β-turn conformation, the γ-lactam constraint as replacement for Gly^6 in the amino acid sequence of LH-RH was examined (Scheme V). The constrained LH-RH analogue (**3**) was found to exhibit a greater potency than that of the parent hormone, for inducing the release of LH both *in vivo* and *in vitro* in adult rats primed with estradiol and progesterone.[20] The constrained LH-RH analogue was later shown to exhibit a Type II′ β-turn conformation.

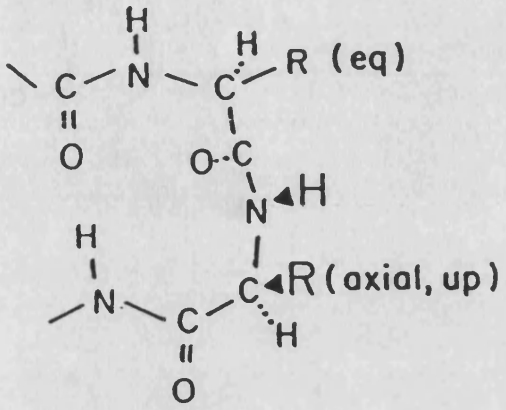
The different types of side-chain to side-chain, and side-chain to backbone linkages, to form cyclic peptides have been reviewed recently by Hruby[3].

1.5 Peptide Mimetics of Secondary Structures and Turns

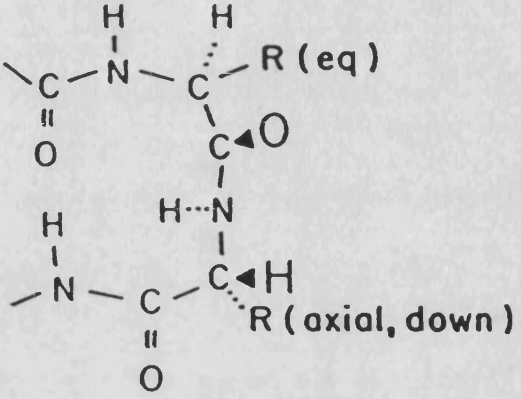
Since folding of polypeptide chains gives rise to the shape of a peptide or protein,[6,21,22] turns in peptides have been proposed to be important loci for receptor binding and transduction, due to their intrinsic polar structure in a compact region of space.[8] Hence, interests have developed in synthesising conformationally restricted peptide analogues of bioactive peptides that will exhibit these turn conformations. Turns are quasi-cyclic structures, where the orientation of side chain groups can be described as either axial or equatorial with respect to the backbone of the turn structure (Figure 11). Bioactivity should therefore not alter provided that the side chain groups are orientated in the same

Fig. II

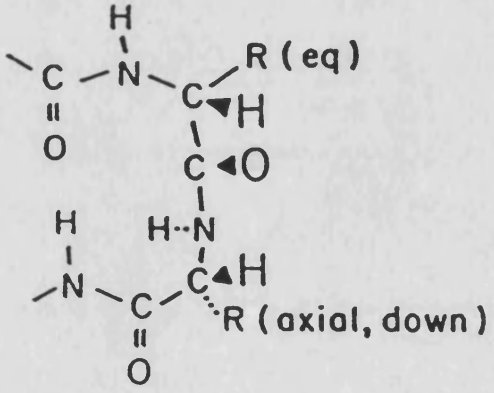
Type I (L-L)



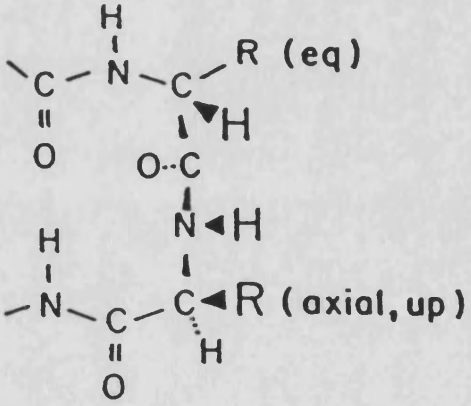
Type II (L-D)



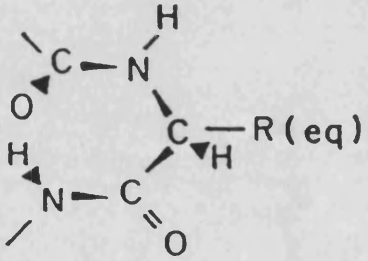
Type I' (D-D)



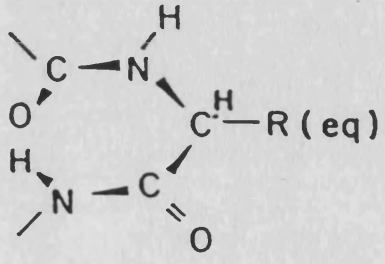
Type II' (D-L)



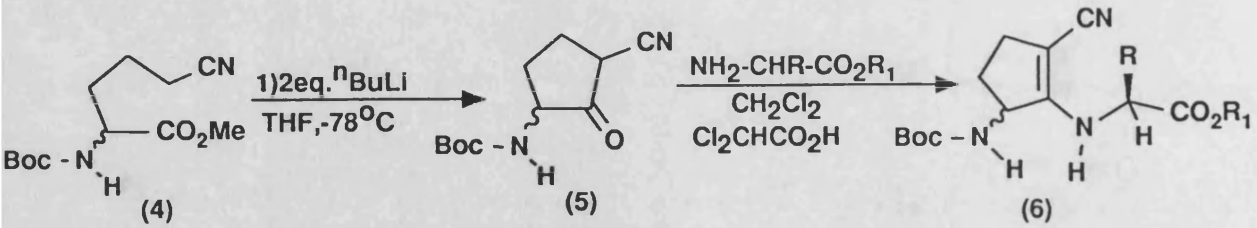
γ-Turn (D)



Inverse γ-Turn (L)



Scheme VII



Scheme VIII

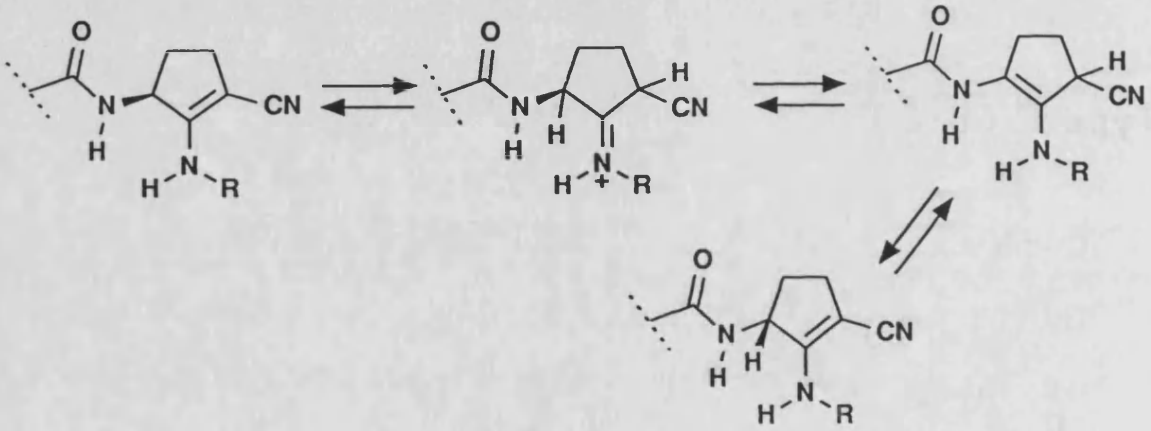
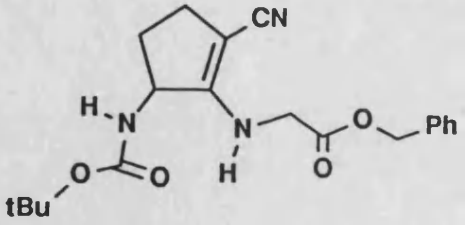
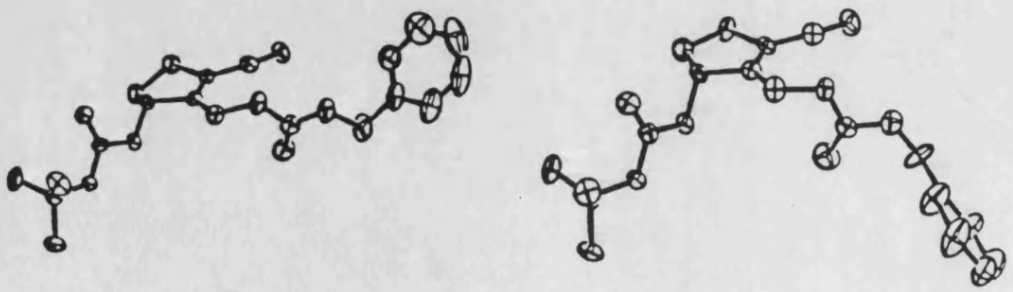


Fig. 12

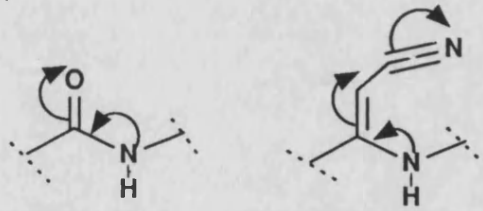




way as in the native molecule. One way of inducing a turn conformation in a peptide chain is by the introduction of pseudopeptide mimetics and this area of work has been reviewed by Morgan and Gainor.[14] This review section will therefore concentrate only on the different types of peptide mimetics that have been applied to induce a γ-turn conformation in a peptide chain.

To date, there have been two reported cases of γ-turn mimetics, compared with the vast number of peptide mimetics that induce or stabilise a β-turn conformation. The first was introduced by Kemp in 1987[23] who reported the synthesis of racemic 1-acylamino-2-aminoalkyl-3-cyano-2-cyclopentene (Mcc) (**6**) (Scheme VII). The enaminonitrile moiety was chosen to mimic the amide bond, because this function is both compact and has a relatively weak tendency to form hydrogen bonds (Scheme VI).

Scheme VI

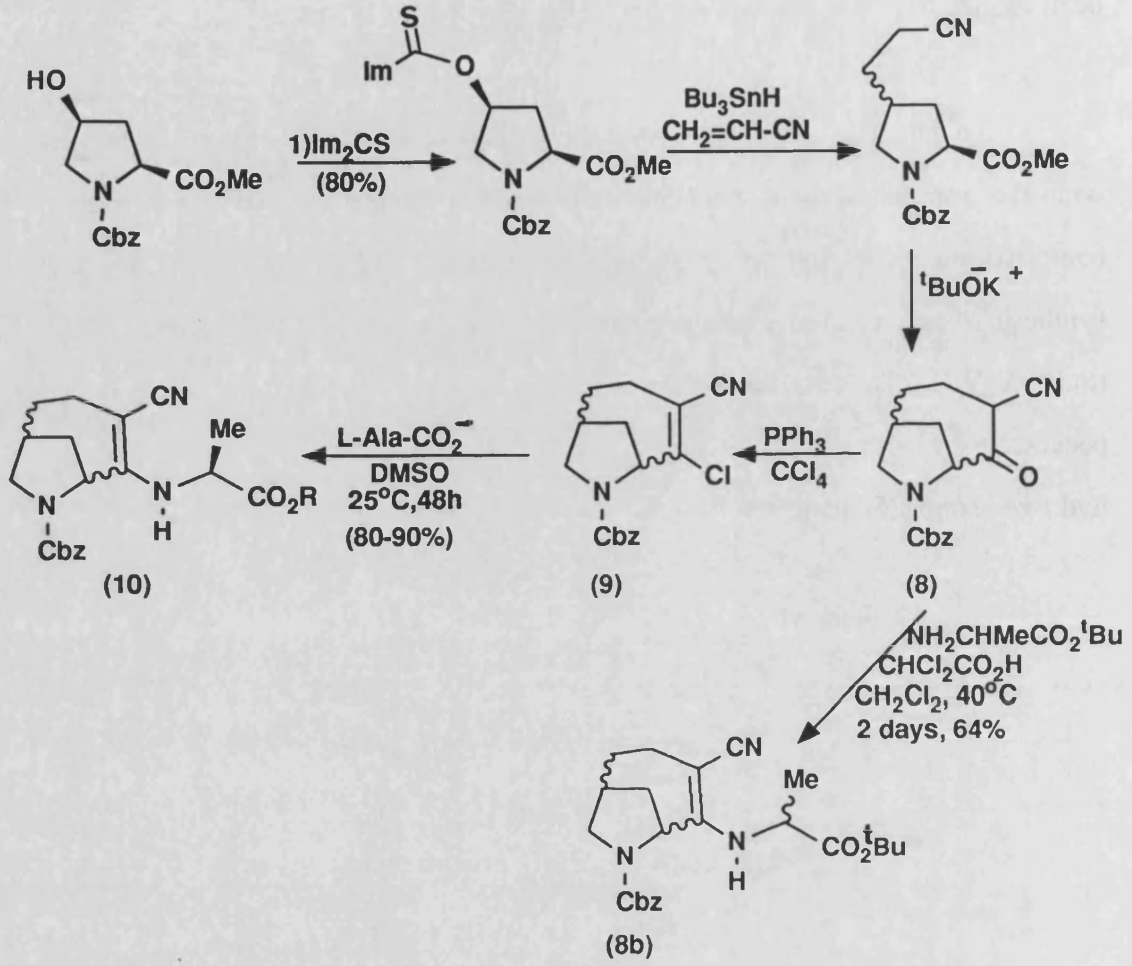


amide bond **enaminonitrile**

Racemic Mcc (**5**) was prepared from methyl-2(N-BOC-amino)-5-cyano-pentanoate (**4**) (Scheme VII) and coupled to amino esters in the presence of the weak acid, dichloroacetic acid. The resulting diastereoisomers were separable by chromatography but were found to epimerise in the presence of trifluoroacetic acid (25°C for 1h) (Scheme VIII). X-Ray crystallographic analysis of diastereoisomer (**7**) revealed two main conformers, which differed by the orientation of the side chain benzyl group and in the puckering of the cyclopentene ring with the β-enaminonitrile function in a near-planar orientation (Figure 12).

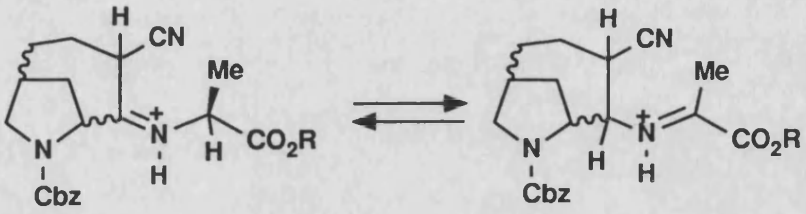
The partial flexibility of Mcc and its ease of epimerisation led to the

Scheme IX


HO
CO2Me
N
Cbz
1)Im2CS
(80%)
S
Im
O
Bu3SnH
CH2=CH-CN
CN
tBuOK
Cl
PPh3
CCl4
O
L-Ala-CO2
DMSO
25°C,48h
(80-90%)
Me
CO2R
H
(10)
(9)
(8)
NH2CHMeCO2tBu
CHCl2CO2H
CH2Cl2, 40°C
2 days, 64%
CO2tBu
(8b)

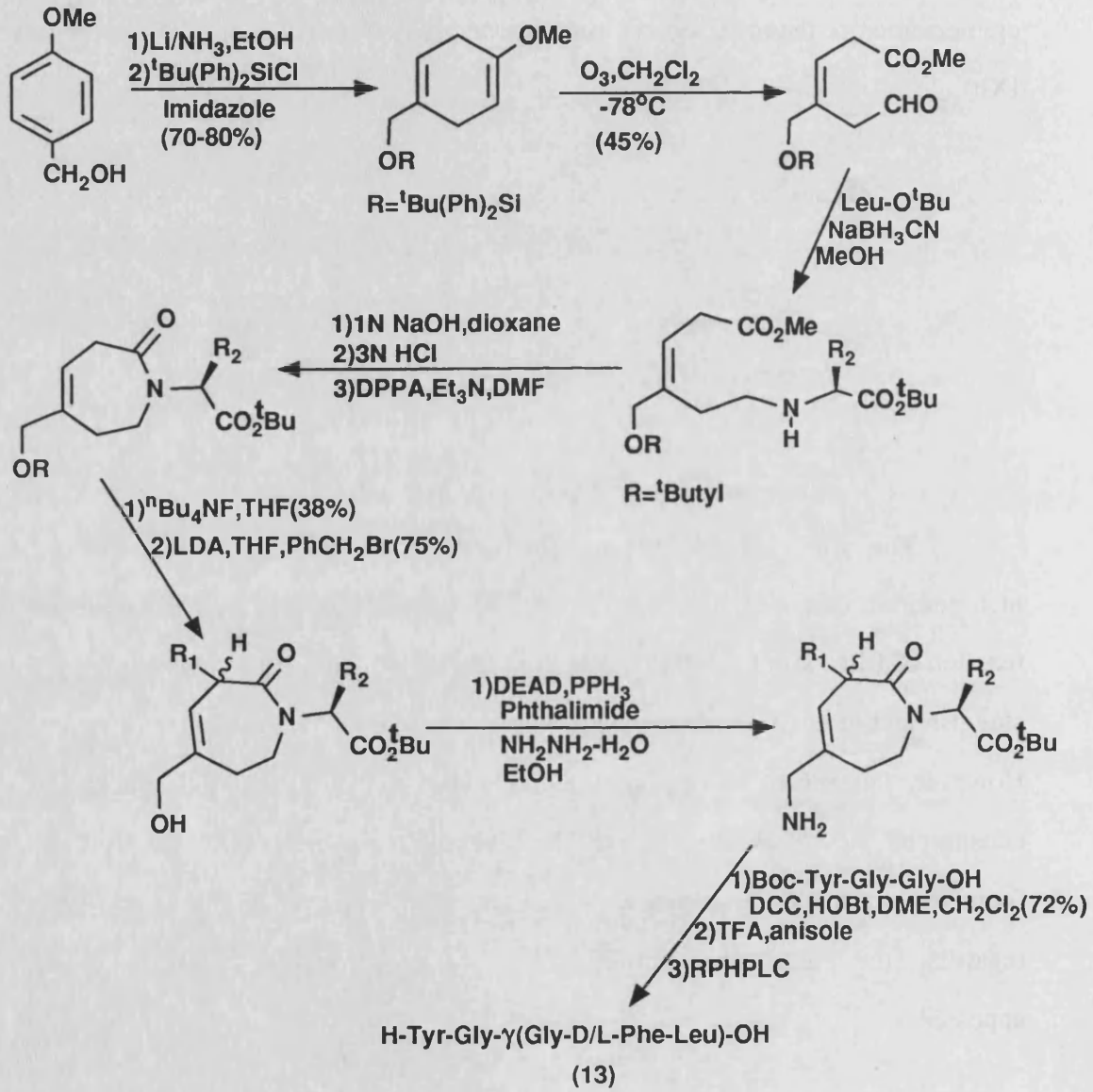
synthesis of the more rigid bicyclic constraint, 4-alkylamino-3-cyano-6--azabicyclo[3.2.1]oct-3-ene (BEN) (**10**), where Bredt's rule prevents the epimerisation process.[24a,b] BEN was prepared from Barton cyano-ethylation of N-Cbz-(2S,4R)-hydroxyproline methyl ester (Scheme IX). This was followed by ring closure in the presence of potassium *tert*-butoxide. The bicyclic α-cyano ketone (**8**) was similarly treated with *tert*-butyl ester of amino acids in the presence of dichloroacetic acid, but because this reaction was slow, competitive epimerisation of the α-carbon centre in the adjacent amino acid occurred (Scheme IXb).

Scheme IXb



The vinyl chloride (**9**) was prepared as an alternative route for the incorporation of amino acids into the BEN skeleton and this was achieved by reaction of BEN with Ph_3P/CCl_4. Reaction of (**9**) with amino acids was, however, sluggish and extensive hydrolysis of this reactive intermediate was also observed. However, this served to demonstrate the ability to couple amino acids to the constrained bicycle BEN, although the dipeptide variant BEN-(L)-Ile-OH was found to be unstable, decomposing on standing in solution. No further publications regarding the use of the bicycle BEN as a conformational constraint have appeared.

Scheme XI

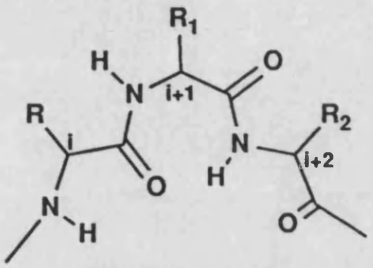


H-Tyr-Gly-γ(Gly-D/L-Phe-Leu)-OH

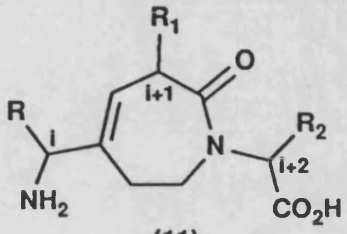
(13)

The second γ-turn mimic to be described in the literature was introduced by Huffmann.[25] A cycloheptene lactam (**11**) was suggested to represent constrained tripeptide analogues of the type γ-(Gly-Gly-Phe), γ-(Gly-D/L-Phe-Leu) and γ-(D,L-Tyr-Gly-Gly), depending on the substituents R, R_1 and R_2 present on the cycloheptene lactam (Scheme X).

Scheme X



General γ turn



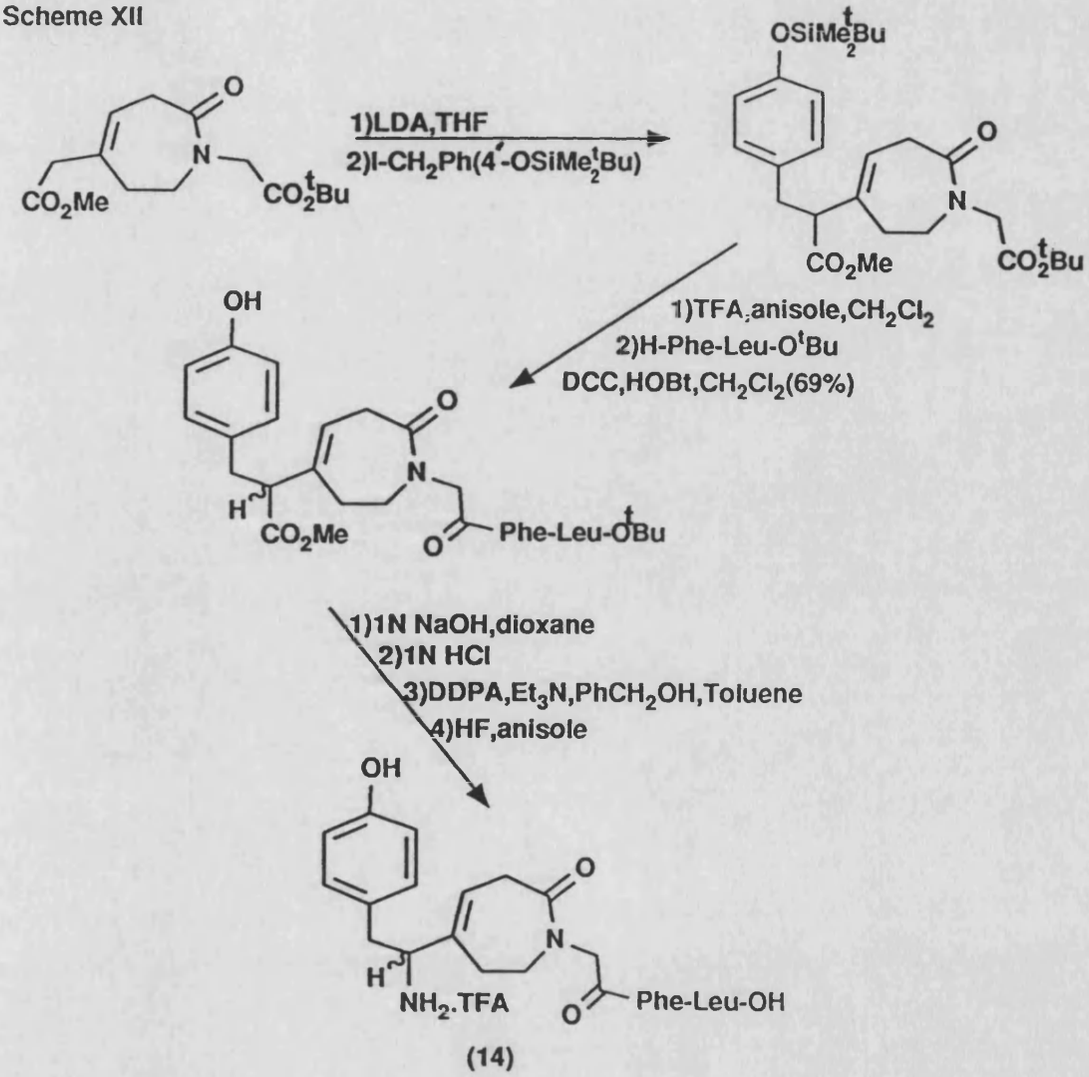
(11)

γ turn mimic

Compound	Name	R	R_1	R_2
(12)	γ(Gly-Gly-Phe)	H	H	Bz
(13)	γ(Gly-D/L-Phe-Leu)	H	Bz	^{t}Butyl
(14)	γ(L,D-Tyr-Gly-Gly)	Bz	H	H

The required cycloheptene lactam was prepared by Birch reduction of 4-methoxybenzyl alcohol, followed by selective ozonolysis of the resulting enol ether. Reductive amination of the intermediate aldehyde, followed by cyclisation, afforded the desired lactam and the selective alkylation strategies that were used to provide the final products containing side chains R and R_1 are shown in Schemes XI and XII.

Scheme XII



(14)

From X-ray crystallographic studies, the stereochemistry about R_1 and R_2 (where R_2=^{i}Pr) were found to correspond to the (S)-configuration. In addition the overall shape of the cycloheptene lactam was virtually identical to that determined theoretically with a variety of potential energy functions. The cycloheptene lactam was found to have torsion angles ϕ and ψ of similar values to that of an ideal γ-turn (Table 2).

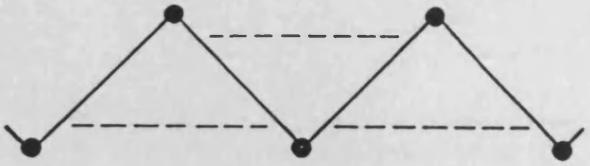
TABLE 2

	ψ_i	ϕ_{i+i}	ψ_{i+i}	ϕ_{i+2}
Ideal γ turn	128°	56°	-67°	-123°
Cycloheptene lactam	120°	80°	-65°	-120°

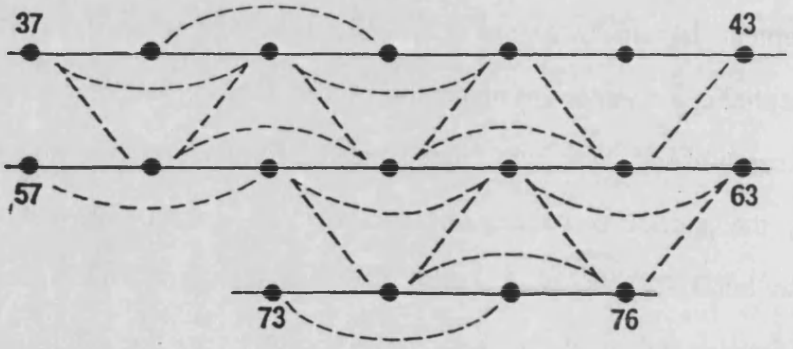
Cycloheptene lactam analogues (**12**), (**13**), (**14**) were then incorporated into leucine enkephalin, an important opioid hormone,[19] as replacements for the Gly^3-Phe^4-Leu^5 subunit and were subsequently tested for their affinity to opiate receptors. Only the peptide containing the cycloheptene lactam (**14**) showed a binding response but this was weak and it was concluded that the bioactive conformation of leucine enkephalin at the μ and δ receptor sites did not contain a γ-turn in the region occupied by these residues.

Very little success in incorporating peptide mimetics at the bioactive site of a peptide hormones or neurotransmitters has been reported, probably due to the fact that most peptide mimetics do not possess appropriately positioned side chain groups.[3]

Fig. 13



2.2_7 Helix



Parallel β Sheet

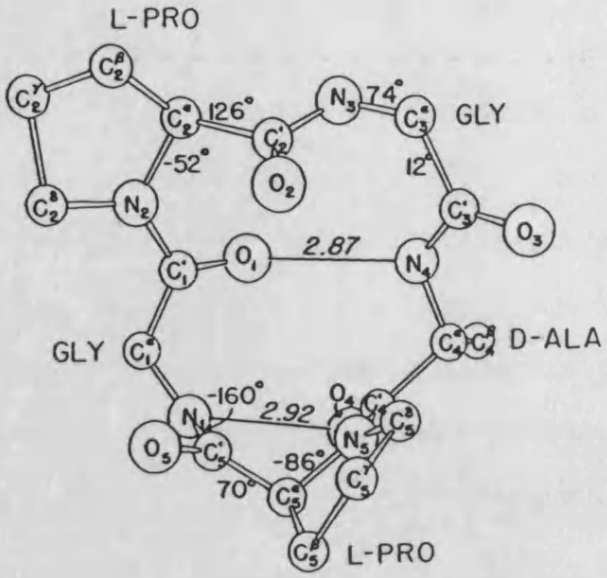
1.6 Occurrence of γ-Turns in Peptides and Proteins

In contrast to the large number of β-turns identified in peptides and proteins, γ-turn conformations have not been widely reported.[7,26] Though Milner-White[27] had published an article on the occurrence of γ-turns in proteins, he concluded that alot more γ-turns (particularly of the inverse type) could be identified if these units were allowed to contain a much weaker hydrogen bond. This was done by calculating the electrostatic energy of hydrogen bonds in 54 known proteins and then grouping those that exhibited a possible γ-turn geometry into two classes, strong and weak.

The strong γ-(and inverse γ) turn had an electrostatic energy >-1.0 kcal mol^{-1} with an interatomic distance of 2.6Å for the crucial N-H--O hydrogen bond and the weaker γ-(and inverse γ) turn had an electrostatic energy of <-1.0 kcal mol^{-1}, with an interatomic N-H--O distance of 3.5Å. The upper limit for a typical hydrogen bond has been judged to be 2.5Å based on estimated van der Waals forces with electrostatic contributions.[7] The weaker inverse γ-turn was found to occur in greater abundance than those of the classic type. These weak inverse γ-turns were found to be present in certain situations in the polypeptide chain. The weaker type tended to occur readily in the middle of strands of β-sheets and exhibited hydrogen bonding similar to that of a 2.2_7 helix (Figure 13). The 2.2_7 helix was first proposed by Huggins in 1943 as a possible conformation for fibrous protein.[28] This secondary structure was described as ribbon-shaped, though not fully extended, with a twist that is almost right handed. The stronger inverse γ-turn tended to be sited at the N or C termini of α-helixes. A number of the stronger inverse γ-turns have been postulated to be at the ligand binding and active sites of several metalloproteins and immunoglobins.

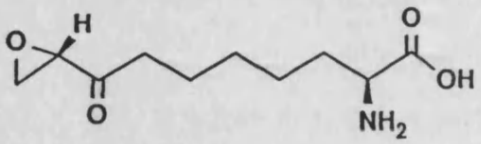
γ-Turns have been implicated to be present in a series of pentapeptides of

Fig. 14



Cyclo-(Pro-Gly-Pro-Gly-D-Ala)

Scheme XIII

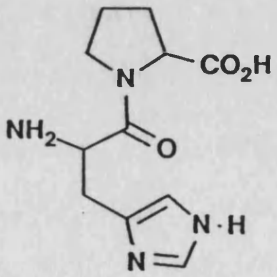


2-amino-8-oxo-9,10-epoxydecanoic acid = Aoe

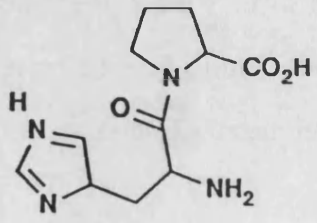
Compound	Sequence
H.C - toxin	cyclo [L-Aoe-D-Tyr(OMe)-L-Ile-L-Pro]
Chlamydocin	cyclo [L-Aoe-Aib-L-Phe-D-Pro]
Wf 3161	cyclo [L-Aoe-D-Phe-L-Leu-L-Pip]
Cyl - 2	cyclo [L-Aoe-D-Tyr(OMe)-L-Ile-L-Pip]

Aib = α-aminoisobutyryl

Scheme IVX



trans-His-Pro



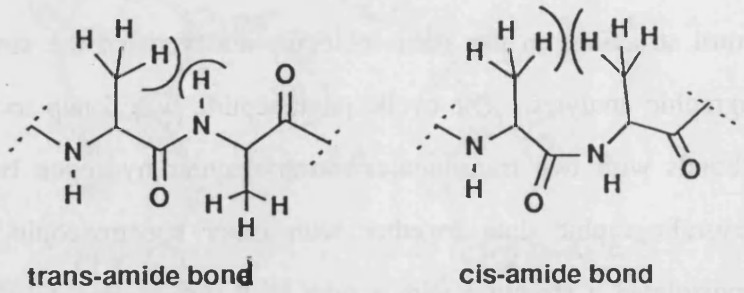
cis-His-Pro

structure *cyclo*-(Xxx-Pro-Yyy-D-Zzz-Pro), according to ^{1}H NMR conformational analysis.[29] Pease and Watson[30] were first to synthesise a cyclic pentapeptide *cyclo*-(Pro^{1}-Gly^{2}-Pro^{3}-Gly^{4}-D-Ala^{5}) which contained the two different types of reverse turn structures in the same molecule and verified the structure by X-ray crystallographic analysis. The cyclic pentapeptide was found to consist of *trans* peptide bonds with two transannular intramolecular hydrogen bonds and, using X-ray crystallographic data together with other spectroscopic techniques, the authors postulated a structure with a type II′ β-turn at Pro^{3}-Gly^{4}-D-Ala^{5}, with a γ-turn at Gly^{1}-Pro^{2}-D-Ala^{5} (Figure 14).

NMR studies of short cyclic polypeptides of four or five residues have also been proposed to manifest γ-turn conformations though none of these features have been reported to be either required or is responsible for bioactivity.[32] A recently published article described the naturally-occurring cyclotetrapeptides H.C-toxin,[33] chlamydocin,[34] WF-3161,[35] and cyl-2,[36] all possessing a peptidyl 12-membered ring with the amino acid, 2-amino-8-oxo-9,10--epoxydecanoic acid (Aoe) as a common feature (Scheme XIII). These peptides have bioactivity both as plant toxins and *in vitro* as cytostatic and antimitogenic agents[37] and various side chain substitutions have established the importance of Aoe for bioactivity.[38] Conformational analysis using various NMR techniques have demonstrated another common structural feature amongst these cyclic tetrapeptides. A *cis-trans-trans-trans* amide bond arrangement has been identified and this has been suggested to be required for bioactivity.[39]

The *s-cis* and *s-trans* isomerism of the His-Pro peptide bond (Scheme XIV) in angiotensin and thyroliberin analogues[40] by ^{1}H and ^{13}C NMR has also been studied and the population of the *s-cis* isomer present in aqueous solution has been correlated with bioactivity.

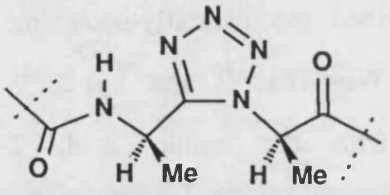
Fig. 15



trans-amide bond

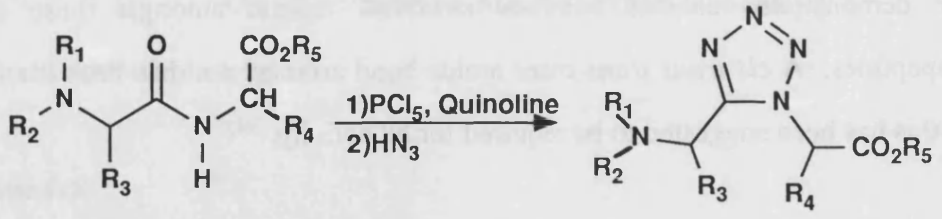
cis-amide bond

Fig.. 16



Tetrazole dipeptide mimetic

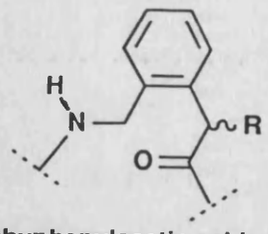
Scheme IVXa



The occurrence of *cis*-amide bonds in peptides are generally not favoured due to steric interactions between adjacent α-carbon substituents[41] (Figure 15). However, this feature has been observed in peptides containing proline[42] or N-methylated amino acids.[43a,b] Recently, a number of bioactive peptides containing a *cis*-peptide bond with neither of the above components have been reported.[44a-c] This has lead to increased interest in the synthesis of constrained peptide analogues that would exhibit a constraint equivalent to a *cis*-amide bond.[45] There have only been two reported cases of peptide mimetics that fix the amide bond in this way. The 1,5-disubstituted tetrazole (CN_4) (Figure 16) has been introduced as a synthetic probe for studies relating to *cis-trans* isomerisation of N-alkylamide bonds, as present in proline. This tetrazole has been successfully incorporated into the dipeptide *cyclo*-[L-Phe-ψ(CN_4)-L-Ala], where the tetrazole ring has been shown to exhibit a strong geometric similarity to the *cis*-amide bond.[46a] The constrained tetrazole was prepared by addition of a dipeptide to a premixed solution of phosphorus pentachloride and quinoline, followed by the addition of hydrazoic acid (Scheme IVXa). Quinoline has been shown to be essential in order to prevent racemisation of the N-terminal amino acid residue.

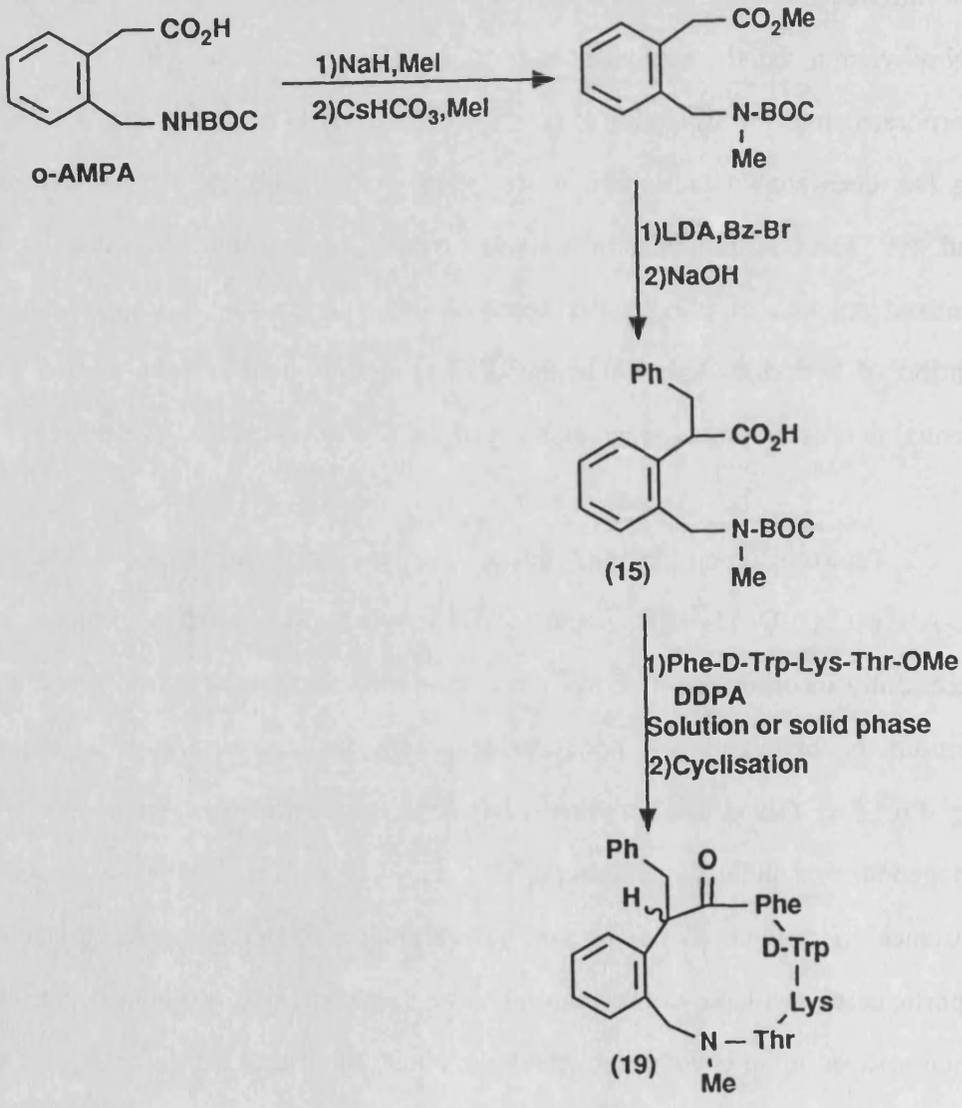
Tetrazole dipeptide mimetics of the type Z-L-Ala^{6}ψ(CN_4)-L-Ala^7-NH_2, Z-L-Ala^{6}ψ(CN_4)-D-Ala^7-NH_2 and Z-L-Pro^{2}ψ(CN_4)-L-Ala^3-NH_2, have been successfully incorporated as constrained *cis*-proline conformers into the 2 and 6 positions of bradykinin, a nonapeptide which has the amino acid sequence Arg^1-Pro^2-Pro^3-Gly^4-Phe^5-Ser^6-Pro^7-Phe^8-Arg^9. Bradykinin is one of the potent endogenous vasolidators and acts both as a depressor and as a myotropic substance. However, as yet no conclusive picture has emerged for the relative importance of available conformational states in relation to biological activity.[8] When assayed upon isolated rat uterus the above three bradykinin analogues were found to be inactive[46b] suggesting that a *cis*-amide conformer at either Pro^2 or Pro^7 is not required, or may even be detrimental for bioactivity.

Fig. 17



o-aminomethyphenylacetic acid = o-AMPA

Scheme XV

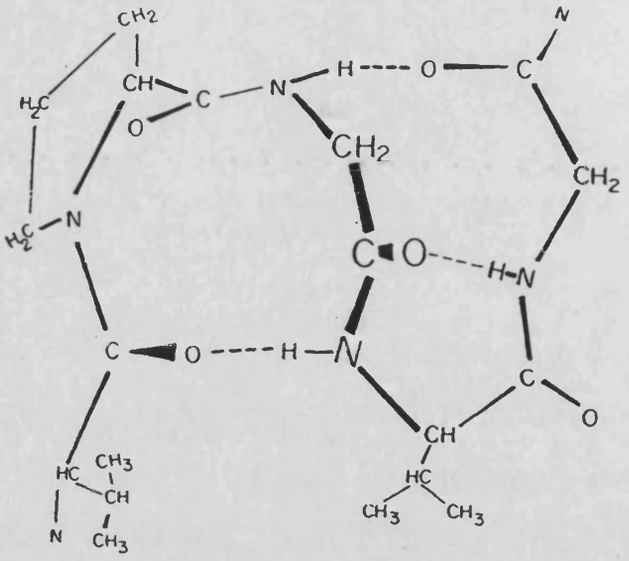


O-Aminomethyphenylacetic acid (o-AMPA) (Figure 17) has been proposed to mimic a *cis*-Gly-Gly unit.[47] A *cis*-amide bond conformation at Phe-Pro had been speculated to be the bioactive conformation for cyclic hexapeptide *cyclo*-(Pro^1-Phe^2-D-Trp^3-Lys^4-Thr^5-Phe^6)[48], a potent analogue of somatostatin for the release of growth hormone. The absence of activity in the peptide *cyclo*-(o-AMPA^1-Phe^2-D-Trp^3-Lys^4-Thr^5) was speculated to be due to the lack of a side chain residue at o-AMPA^1-Thr^5 and by the presence of a strong hydrogen bond between the NH and the carboxyl group in o-AMPA. The aminomethylene group was then N-methylated, followed by alkylation at the α-position of the carboxyl group in the o-AMPA (Scheme VX). Incorporation of this new constrained dipeptide unit (**15**), into the peptide chain (Phe-D-Trp-Lys-Thr), followed by cyclisation using diphenylphosphoryl azide, afforded the *cyclo*-peptide (**16**) as two separable diastereoisomers. While devoid of any biological activity *in vitro,* one diastereoisomer significantly inhibited the release of growth hormone *in vivo*, with an IC_{50} of 52μg/kg *vs* 62μg/kg for that of the parent somatostatin analogue. Here a much better case can be made for the requirement of a *cis*-peptide bond in the bioactive conformation of cyclic somatostatin analogues.

Given the published reports and reviews of turns in peptides, only bioactive peptides that have been postulated to exhibit a γ turn conformation in solution, will be summarised here.

Pentapeptide Arg^1-Pro^2-Asp^3-Val^4-Tyr^5 ([Pro^2]TP5), a biologically active analogue of the immunoregulatory peptide thymopentin, Arg^1-Lys^2-Asp^3--Val^4-Tyr^5 (TP5), has been proposed to exhibit a Type II β-turn involving the first four residues with a γ-turn at Asp^3.[49] Similarly, a β- and γ-turn have been reported for elastin, a polypentapeptide of amino acid sequence

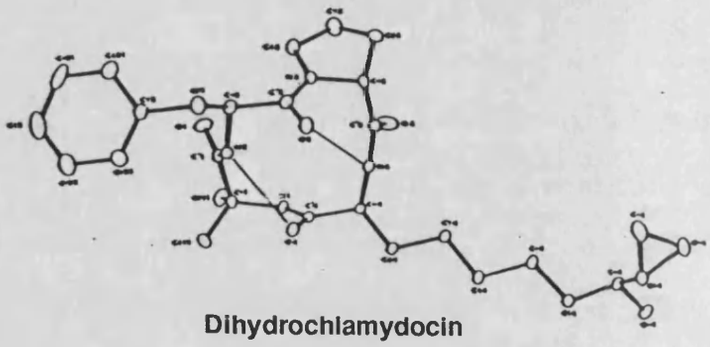
Fig. 18



Polypeptide of Elastin

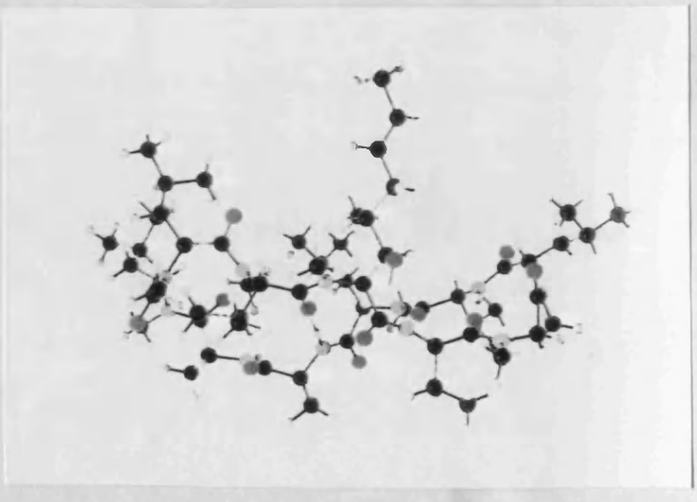
$HCO(Val\text{-}Pro\text{-}Gly\text{-}Val\text{-}Gly)_n\text{-}Val\text{-}OMe$

Fig. 19



Dihydrochlamydocin

Fig. 20



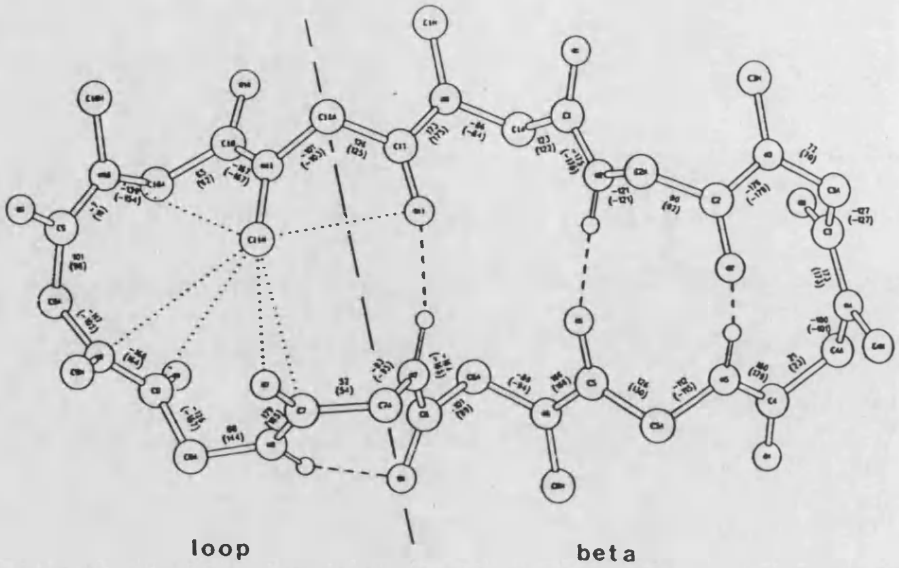
HCO-(Val[1]-Pro[2]-Gly[3]-Val[4]-Gly[5])$_n$-Val-OMe where n=18. Two β-turn structures are believed to be present between residues Val[1]-C=O with Val[4]-NH (with 10 atoms in the turn) and Gly[3]-NH with Gly[5]-C=O (with 11 atoms in the turn) with a γ-turn at Gly[3]-C=O with Gly[5]-NH[(50)] (Figure 18).

X-Ray crystallographic data of γ-turns in bioactive peptides are however scarce and to date there are only two reported cases. The first was described by Flippen and Karle[(51)] in the cyclic tetrapeptide dihydrochlamydocin, an analogue of the cytostatic peptide chlamydocin.[(34,37)] Dihydrochlamydocin has an amino acid sequence *cyclo*-(Aoe[1]-DiMe-Gly[2]-Phe[3]-D-Pro[4]) and is characterised by two γ-turns, together with four transoid amide bonds. This latter feature is so-called because the amide bonds are twisted by between 15° to 25° from planarity. Solution conformational analysis of chlamydocin, as determined by NMR techniques, was found to be in close agreement with that found by X-ray crystallographic analysis of dihydrochlamydocin (Figure 19).[(52)]

Cyclosporin A isolated from fungus species *Tolypocladium inflatum Grams*[(53)] is an undecapeptide containing seven N-methylated amino acids and is a potent immunosuppressent, used clinically to suppress the rejection of transplanted human organs.[(54)] The amino acid sequence was first determined by X-ray crystallographic analysis of the iodo-derivative of cyclosporin A.[(55)] The peptide was shown to contain an anti-parallel β-pleated sheet together with two intramolecular hydrogen bonds representing a β- and a γ-turn. The overall shape of iodo-cyclosporin A has been described to be butterfly-shaped with the unsaturated amino acid, N-methyl-4-butenyl-4-methylthreonine (MeBmt), protruding out like that of an insect's proboscis (Figure 20).

The structure of cyclosporin A was later refined and verified by Loosli[(56a,b)] using X-ray and solution conformational analysis. Two structural

Fig. 21



Cyclosporin A

features in cyclosporin A was revealed. The β-fragment, containing residues 11 to 7, formed an anti-parallel β-pleated sheet and a Type II′ β-turn at residues 3 and 4. Residues 7 to 11 formed the second structural feature, the so-called "loop". The "loop" was found to contain a γ-turn involving D-Ala^8 and Me-Leu^6 and a *cis*-amide bond was found between Me-Leu^9 and Me-Leu^{10} (Figure 21). There are, however, a few discrepancies regarding the backbone conformation of cyclosporin A. A C_5 bend between the NH-D-Ala^8 with the adjacent peptide bond was indicated by NMR, but this feature was not observed in the solid state. The orientation of the side chain amino acid (MeBmt) was also found to be different to that predicted from solution phase analysis i.e. χ=+60° (in solution) compared to χ=-168° (in the solid state). The presence of MeBmt has been demonstrated to be important for the immunosuppressive activity of cyclosporin A[57a-e] and subsequent analogues of this side chain amino acid have been examined.[58-60] The application of a δ-lactam constraint to the β-turn in cyclosporin A has been investigated[61] but only weak immunosuppressive biological activity was observed.

Recent NMR studies[62] of substance P, neurokinin A and B have all suggested that there is a common γ-turn structure in the peptide chain. These peptides are members of the trachykinin family and are neuropeptides that possess a similar C-terminal amino acid sequence (Table 3).

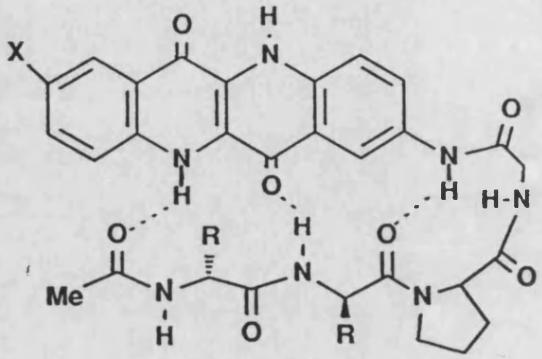
Table 3

Substance P	Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2
Neurokinin A	His-Lys-Thr-Asp-Ser-Phe-Val-Gly-Leu-Met-NH_2
Neurokinin B	Asp-Met-His-Asp-Phe-Phe-Val-Gly-Leu-Met-NH_2

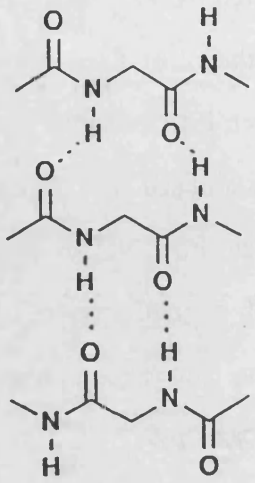
These peptides are of considerable interest due to their putative activity as neurotransmitters of pain and substance P and neurokinin A have also been shown to stimulate inflammation and vasolidation. A neurokinin antagonist is thus potentially useful as a non-narcotic analgesic and as an anti-inflammatory agent. Several neurokinin anagonists have since been prepared and this area has been reviewed by Vaught[63] and by Logan.[64] NMR studies of substance P carried out in methanol by Chassaing and coworkers,[65] suggested an α-helical conformation between Pro^4 and Phe^8. This structure is thought to be comprised of a 1.5 helical turn, stabilised by hydrogen bonds between Phe^7-NH and O=C-Lys^3 as well as between Phe^8-NH with O=C-Pro^4, with a proposed γ-turn involving Leu^{10}. Indeed, a small amount of α-helix was also detected by Wu and Yang (in 25mM sodium dodecyl sulphate (SDS) solution) using circular dichroism spectrospcopy.[66a-c] Similarly, neurokinin A has been reported to possess a γ-turn at sequence Lys^2-Asp^4, stabilised by an internal salt bridge (His^1 or Lys^2 to Asp^4) from NMR studies in DMSO.[67] Loevillet has explored the conformation of neurokinin B and has suggested the presence of a γ-turn at Met^2 and that this structure was stabilised by a salt bridge between the carboxylate Asp^1 and the imidazole of His^3.[62]

Although these results are speculative as conditions and the methods used were widely diverse, they nevertheless demonstrated the need for the different types of constrained units required to examine the different types of turns that are available in a bioactive peptide. This is especially true for γ-turns, since these are now being increasingly recognised to be present in bioactive compounds.

Fig. 22

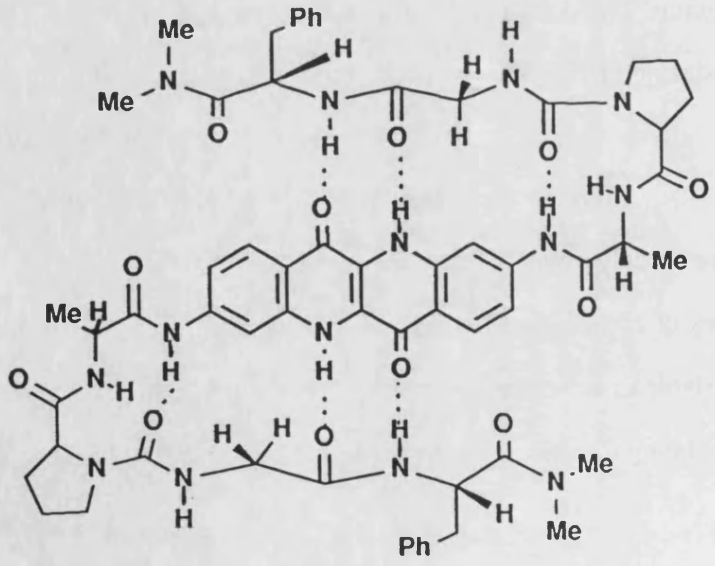


(17)



Parallel β Sheet

Fig. 23



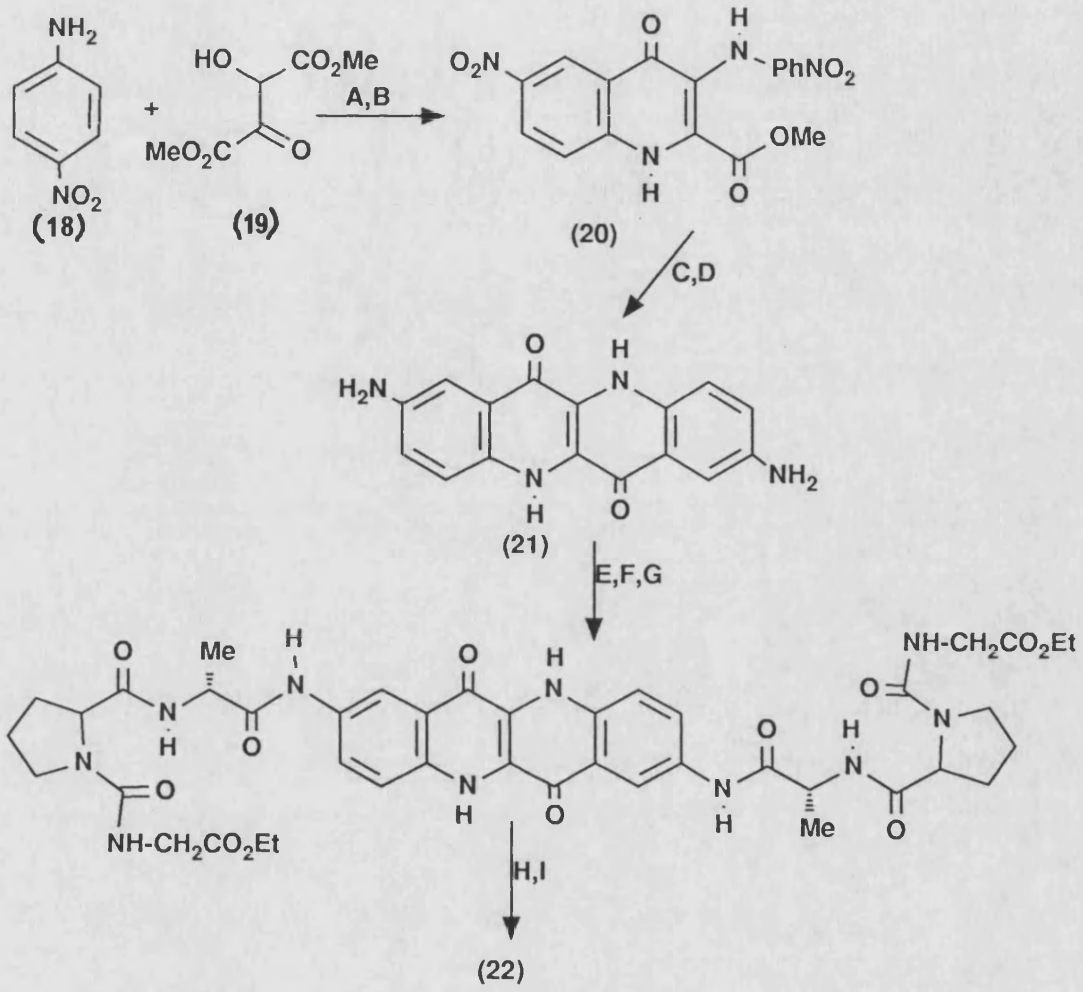
(22)

1.7 The Use of Peptide Mimetics to Initiate Folding in Polypeptide Chains

Hydrogen bonds in secondary structures have been thought to act as "seeds" for the folding of peptides.[68] By understanding their role in protein folding it may ultimately lead to the design and synthesis of new proteins with tailor-made structural and functional properties. Recent achievements in protein design have been reviewed by Mutter and Vuilleumier[69] and only peptide mimetics that have been designed to initiate folding (i.e. α-helix or β-sheet formation) in peptides will be discussed in this chapter.

Two different types of secondary structural peptide mimetics, corresponding to a β-sheet and an α-helix, have been introduced by Kemp and co-workers.[70] Epindolidinone-peptide, containing a β-turn conformation (D-Ala-L-Pro), is thought to act as a template for β-sheet formation.[71,73] A reversal of peptide bonding sequence at the 2-position of epindolidinone was recognised and this should give rise to a characteristic hydrogen bonding pattern similar to that of a parallel β-sheet (**17**) (Figure 22). As a route to anti-parallel β-sheet formation, an urea functionality was introduced into the peptide sequence to counteract this peptide bonding reversal (Figure 23). The overall synthesis of epinolidinone-peptide, *bis*-2,8[(N-ureido-Gly-L-Phe-NMe_2)-L-Pro-D-Ala-NH)]-epinolidinone (**22**) is outlined in Scheme XVI. Dimethyl dihydroxyfumarate (**19**) was reacted with 4-nitroaniline (**18**) to give the carbomethoxyquinolone (**20**). Thermal cyclisation generated 2,8-diamino-epinolidinone (**21**) and this diamine was then coupled with the corresponding amino acids (D-Ala-Pro), followed by ethyl isocyanoacetoacetate and Gly-L-Phe-NMe_2 to give the template-peptide (**22**). ^1H NMR conformational analysis on epinolidinone-peptide (**22**), established the structure as an anti-parallel β-sheet with a Type II′ β-turn conformation.[72,73]

Scheme XVI



A; HCl, MeOH (64%): B; Dowtherm A,15 min 250°C (55%);

C: $SnCl_2$, HCl; D: $AlCl_3$-NaCl, 160°C, 1h;

E: Et_3N, $(BOC\text{-}D\text{-}Ala)_2O$, DMSO (97%);

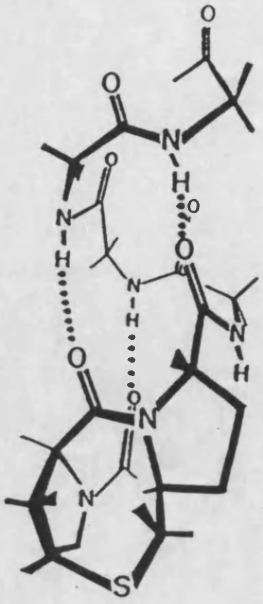
F: TFA, then Et_3N, BOC-L-Pro-OC_6F_5, in DMF (92%);

G: TFA, then Et_3N, O=C=N-CH_2CO_2Et in DMF (61%);

H: LiOH, THF-H_2O (79%);

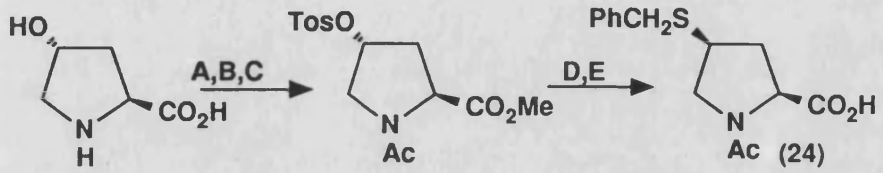
I: $^iPrN{=}C{=}N^iPr$, HOBt, H-L-Phe-NMe_2, DMF (71%)

Fig. 24

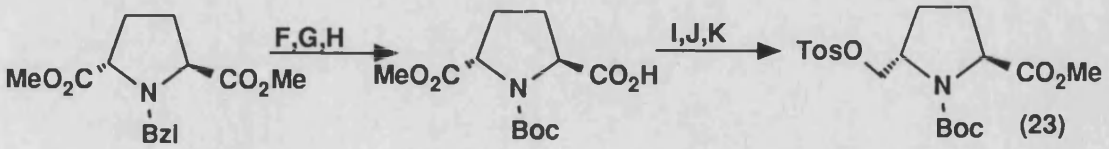


The α-helix peptide mimetic, (2S,5S,11S)-1-acetyl-1,4-diaza-3-keto-5-carboxy-10-thiatricyclo[2.8.0]tridecane (**26**) has been specifically designed and synthesised with the three amide carbonyls having the same pitch and spacing as that present in an α-helix.[74,76] This cluster was designed to act as the nucleation site to initiate α-helix formation when attached at the N-terminus of a linear polypeptide chain (Figure 24). The tricycle template was prepared by condensation of *trans*-1-(*tert*-butyloxycarbonyl)-2(S)-(methoxycarbonyl)-5(S)--[(tosyloxy)methyl]pyrrolidine (**23**) with 1-acetyl-2(S)-carboxy-4(S)-thio-pyrrolidine (**24**) to give the thioether (**25**), which was then cyclised under high dilution conditions (pyridine, 80°C) *via* the active 4-nitrophenyl ester (Scheme XVII).[74,76]

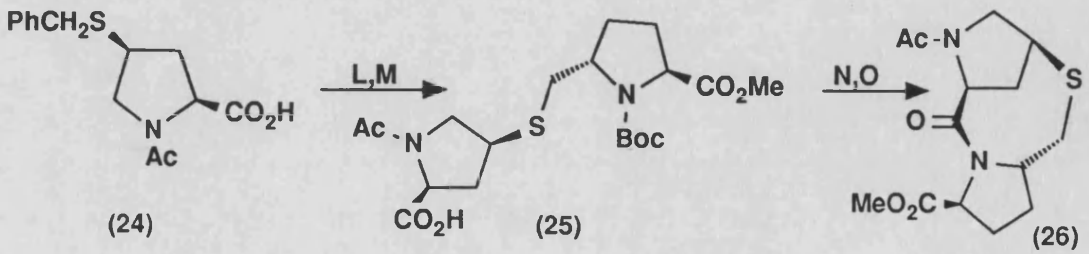
Scheme XVII



A: $SOCl_2$,MeOH (75%); B: Ac_2O,$NaHCO_3$-H_2O-dioxane (78%);
C: Tos-Cl,py (64%); D: NaOH,H_2O (87%); E: $PhCH_2SNa$,DMSO (72%).

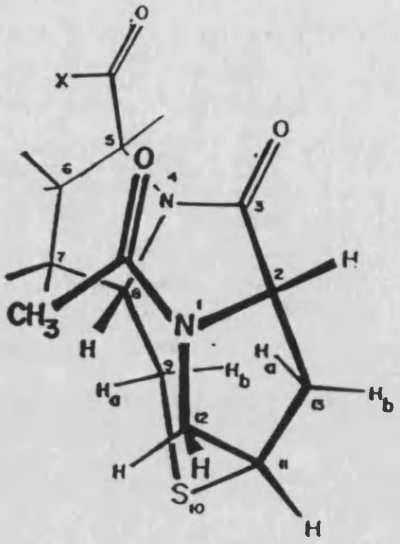


F: H_2/Pd EtOH (91%); G: BOC_2O,MeCN (90%); H: NaOH,H_2O;
I: $LiBH_4$; J: CH_2N_2 (44%)(H thru J); K: TosCl,py (83%)

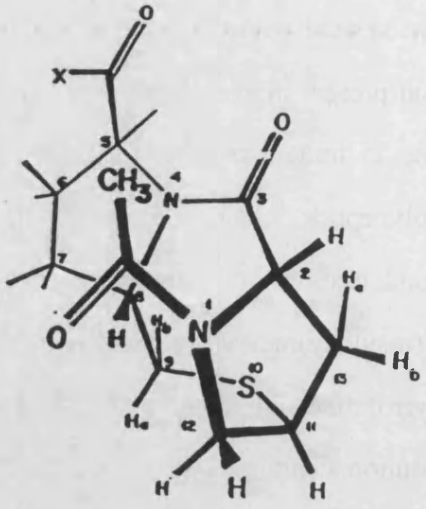


L: Na/NH_3; M: (23) in DMSO (73%); N: p-NO_2-C_4H_4OH,DCC,CH_2Cl_2 (85%);
O: TFA,then 10^{-3}M in py-dioxane,100°C (20%).

Fig. 25

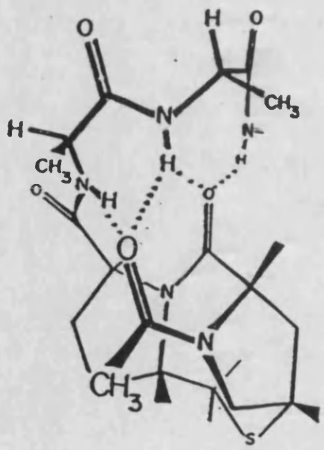


s-trans



s-cis

Fig. 26



Bifurcated H-bonding

^{1}H NMR conformational analysis of the tricyclic unit (26) found two principle conformations in solution, corresponding to the *s-cis* and *s-trans* 1-acetamido function (Figure 25). The *s-trans* conformation exhibited nucleating properties, in that all three carbonyls are aligned in a helical array and the sulphur atom is positioned downwards facilitating hydrogen bonding to the acetamide carbonyl. In the *s-cis* conformer, the sulphur atom is positioned upwards and the acetoamido function is incapable of helical hydrogen bonding. This was thus assigned as the non-nucleating conformer. These results were in accordance with X-ray crystallographic analysis of the tricyclic acid, where the *s-cis* conformer was isolated.

Incorporation of the tricyclic template (26) into a series of peptide chains of the type, α-Temp-L(Ala)$_n$-OR (where n = 1 to 6, R = Me or ^{t}Bu and Temp = tricyclic template) and α-Temp-L-Ala-L-Phe-L-Lys-(ϵBoc)-L-Lys-(ϵBoc)-NHMe (where ϵ indicates an unknown configuration at an asymmetric centre), have been studied by ^{1}H NMR for the presence of a helical conformation. The template-tetrapeptide complex was found to exhibit a distorted α-helix structure with a bifurcated hydrogen bond[7] in acetonitrile[75] (Figure 26). For α-Temp-(L-Ala)$_n$-OR (n = 3,4,5 and 6) a helical conformation was observed in weakly polar organic solvents, such as chloroform and acetonitrile, whereas in more polar solvents (dimethylformamide and dimethylsulphoxide) a mixture of helical and non-helical peptides were observed. This was suggested as evidence that the attachment of the template to the alanine sequence serves to nucleate a helical structure from the N-terminus of the peptide chain. The helical nucleating capacity of the template was, however, destroyed by the addition of N-methylglycine at the junction between the template and the alanine amino acid sequence.[76]

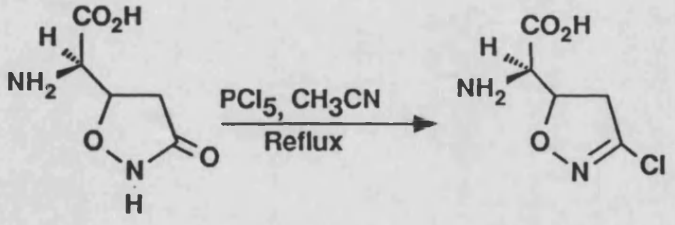
Nucleation from the C-terminus of monomeric peptide chains of <20 units

has recently been published.[77] Side chains of histidine and cysteine residues at positions i and i+4 of the peptide were used to interact with transition metal ions leading to a bidentate complex and which concurrently fixed the peptide backbone into an α-helix conformation.

2.0. Aim of the Project and Proposed Routes

Our own approach to the synthesis of constrained heterocyclic units that would "induce" either a γ-or an inverse γ-turn in a peptide chain, is based on the heterocyclic skeletal structure of 4-amino-3-isoxazolidinone, more commonly known as cycloserine. We envisaged the incorporation of an imidoyl halide (chloride) moiety within the 3-isoxazolidinone ring based on chemistry already established by workers involved in the synthesis of AT-125 from tricholomic acid[78-80] (Scheme XVIII).

Scheme XVIII

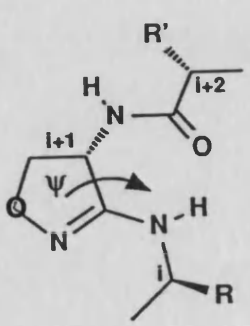


Tricholomic acid **AT-125**

Kemp and Carter[23-25] have already demonstrated the conversion of a carbonyl bond to a vinyl chloride in the bicyclic substrate 3-cyano--6-azabicyclo[3.2.1]octan-3-one (Section 1.5), using triphenylphosphine and carbon tetrachloride and the subsequent displacement of chloride with *tetra*-methylammonium salt of various amino acids.

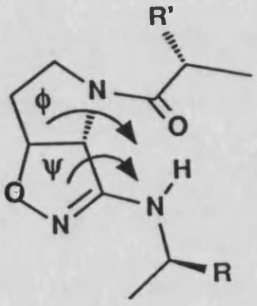
Cycloserine should also provide a means of coupling amino acids to the

Scheme XX



(S)-I

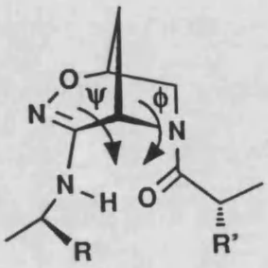
ψ = +60°



(S)-II

ϕ = -65.9°

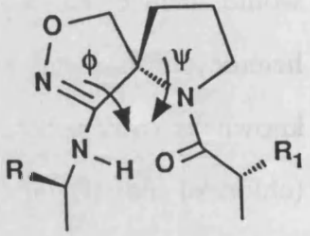
ψ = +62.2°



(S)-III

ϕ = -95.8°

ψ =+113.9°



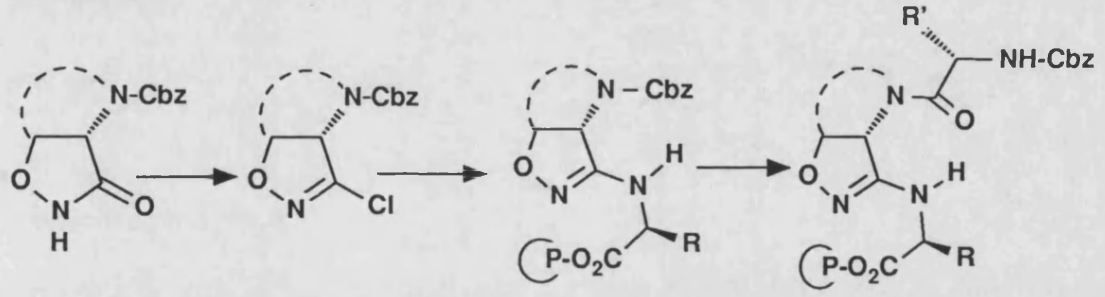
(S)-IV

ϕ = -60°

ψ = +60°

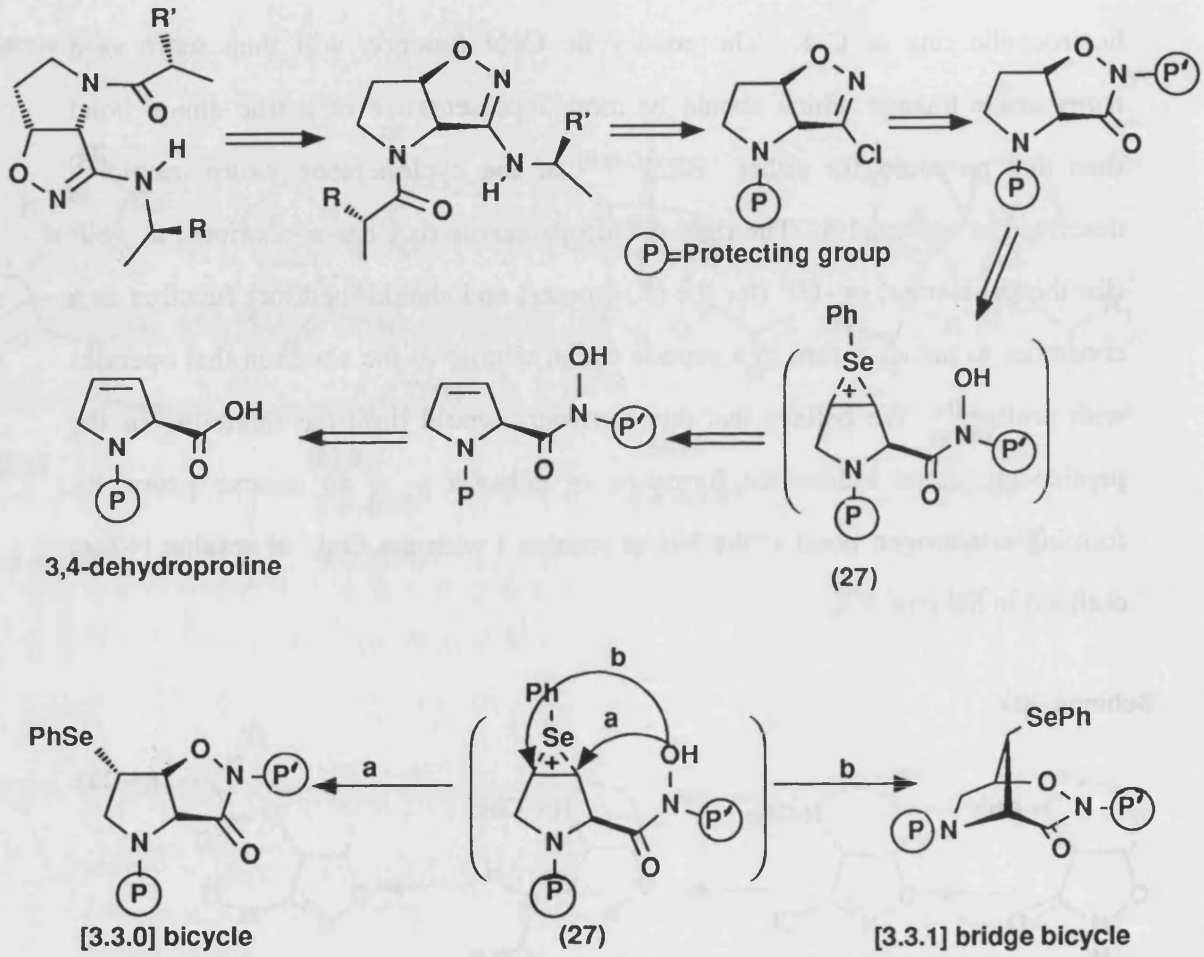
dihydroisoxazole ring in both directions. By displacement of the chloride with the amino terminus of an amino acid (or peptide) generates one chain. In addition, coupling of the amino group at C-4 of the dihydroisoxazole ring to the carboxylic acid terminus of an amino acid (or peptide) would allow the peptide to be extended in the other direction (Scheme XIX). The dihydroisoxazole ring can mimic either a constrained (L)- or (D)-glycine unit, depending on the stereochemistry of the heterocyclic ring at C-4. The endocyclic C=N function will then serve as a *trans*-amide linkage which should be more representative of a true amide bond than that proposed for either BEN[24a,b] or the cycloheptene γ-turn mimic[25] described in section 1.5. The rigid dihydroisoxazole ring has ψ restricted to +60° (for the (S)-isomer) or -60° (for the (R)-isomer) and should therefore function as a constraint to induce a turn in a peptide chain, similar to the situation that operates with proline.[8] We believe that this constraint would limit the flexibility of the peptide chain and induce the formation of either a γ- or an inverse γ-turn, by forming a hydrogen bond at the NH of residue i with the O=C of residue i+2 as outlined in Scheme XX.

Scheme XIX



The chemistry developed with the cycloserine ring system could be applied to more rigid bicycles **II, III** and **IV** (Scheme XX). These constrained units could then be similarly incorporated into a peptide chain (Scheme XIX). Bicycle (**II**) (a *cis*-fused cycloserine unit) mimics a constrained (L)-glycine unit, where both ϕ and ψ torsion angles are more closely restricted to values similar to

Scheme XXI


R'
R
H
O
N
Cl
P
P'
=Protecting group
Ph
Se
OH
3,4-dehydroproline
(27)
a
b
PhSe
SePh
[3.3.0] bicycle
(27)
[3.3.1] bridge bicycle

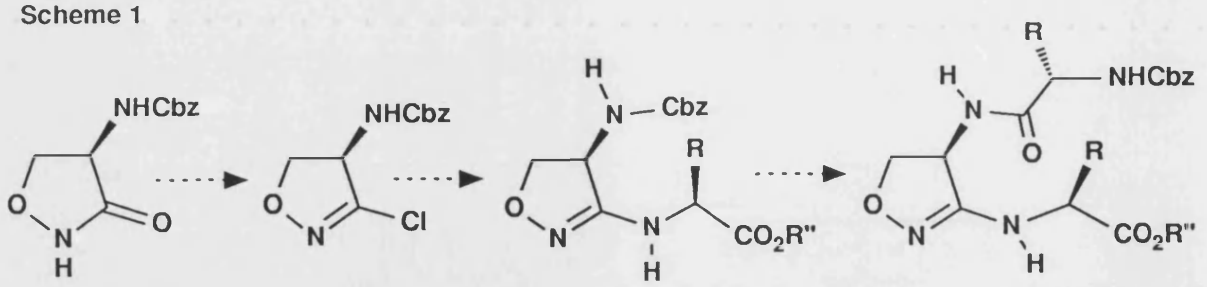
those of an inverse γ-turn (Table 1).

Retrosynthetic analysis of the heterocyclic constrained bicycle (**II**) suggested 3,4-dehydroproline as a convenient starting material (Scheme XXI). We also envisaged the formation of the [3.2.1]bridged bicycle (**III**) from the electrophile-mediated cyclisation of N-benzylhydroxylamine derivative of 3,4-dehydroproline with phenylselenenyl chloride (**27**). The torsion angles of bridged bicycle (**III**) have been calculated (using CONCORD and optimised using the CHEMX molecular mechanics package (20 cycles per sec, dielectric constant=4)) to be ϕ=-95.8° and ψ=+113.9°. Bicycle (**III**) should therefore provide a novel type of conformational constraint and was targeted for this reason.

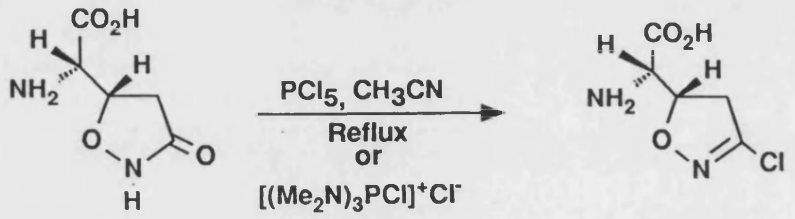
Finally, the proposed (S)-*spiro*-cycloserine unit (**IV**) has been demonstrated (from theoretical studies) to have torsion angles ϕ=-60° and ψ=-60° which corresponds to a right-handed α-helix. If bicycle (**VI**) could be successfully incorporated into a peptide chain, this unit could be used not only as an α-helix constraint but also to induce a helical formation from the middle of the peptide chain and not simply from either of the C- or N-terminus of the peptide [70,77].

RESULTS AND DISCUSSION

Scheme 1



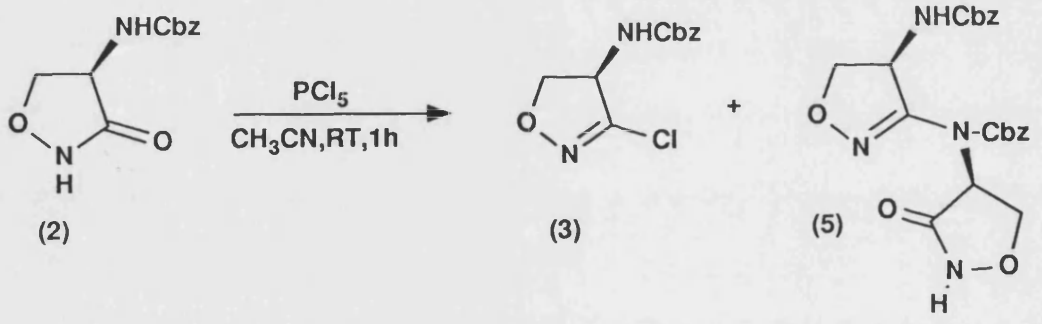
Scheme 2



Tricholomic acid

AT-125

Scheme 3

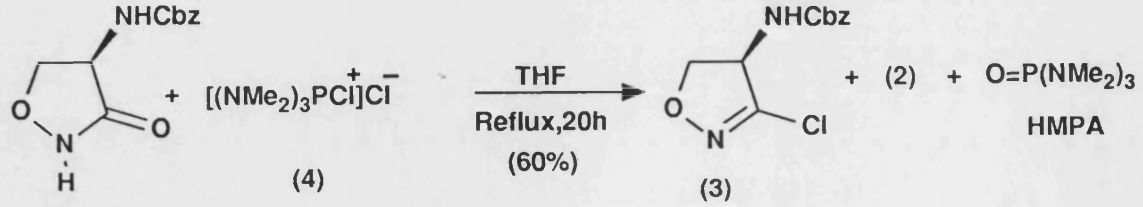


(2)

(3)

(5)

Scheme 4



(2)

CHAPTER 1

1. Synthesis and Reactivity of 3-Chloro-(4R)-Amino-4,5--Dihydroisoxazole with Amine Nucleophiles

1.1. Preparation of 3-Chloro-(4R)-Amino-4,5-Dihydroisoxazole

The initial challenge presented by this programme was to convert the carbonyl moiety in (R)-cycloserine (**1**) to an imidoyl chloride (*see Introduction, section 2.0*) which was seen as suitable handle for the incorporation of this molecular constraint into a peptide chain by direct displacement of this reactive functionality with amino acids (Scheme 1).

Two different methodologies have been reported for the conversion of a carbonyl to an imidoyl chloride within a dihydroisoxazole ring (Scheme 2). The first was developed by Baldwin and coworkers in the synthesis of AT-125 ((αS,5S)-α-amino-3-chloro-4,5-dihydro-5-isoxazoleacetic acid) from tricholomic acid.[78] Here, phosphorus pentachloride was used to activate the carbonyl function and chlorinate it in an addition-elimination fashion. The second method described in the literature utilised dichloro*tris*(dimethylamino)phosphorane (**4**) as the chlorinating agent for the conversion of tricholomic acid to AT-125.[79,80]

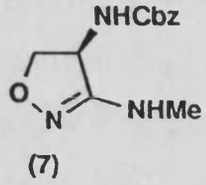
We envisaged 3-chloro-4-amino-4,5-dihydroisoxazole (**3**) to be a suitable substrate for the investigation of the key nucleophilic displacement reaction. Our first attempt to prepare this substrate was by treatment of (4R)-[N-(Cbz)]-cycloserine (**2**) with PCl_5 in nitromethane at reflux but this afforded a complex mixture of products. Repeating this reaction under milder conditions (stirring at room temperature) gave a mixture of the desired 3-chloro-(4R)-[N-(Cbz)amino]-4,5-dihydroisoxazole (**3**) together with dimeric

Table 1

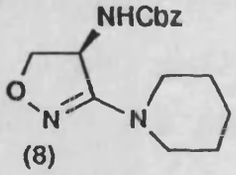
Nucleophile	Temp/°C	Rxn Time/h	Adducts	Yield /%
Xs.Methylamine	95	22	(7)	44
Xs.Piperidine	110	18	(8)	73
1eq.Benzylamine	120	72	(9)	51
Xs.Benzylamine	120	7	(9)	50
Xs.Ammonia	110	48	Decomposition	
Xs.Tryptamine	100	24	(10) (3) (6)	48 30 7
*Xs.Tryptamine	100	48	(55) (54) (55b)	52 34 9
Xs.(L)-Ala-7-Me-Tryptamine	100	7 days	(3) (6)	45 25

* reaction with (S)-Cbz-cycloserine

Xs = excess



(7)



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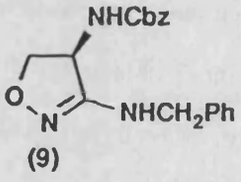

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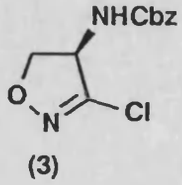

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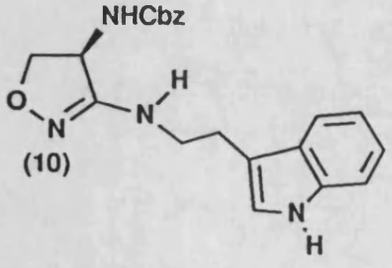

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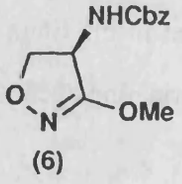
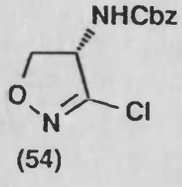


(6)



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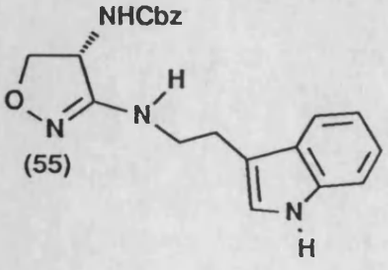

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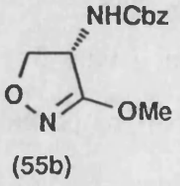
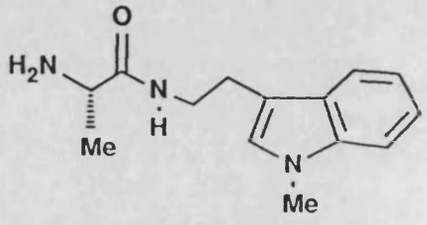


(55b)



(L)-Ala-7-Me-tryptamine

product (4R)-[N-(Cbz)]-cycloserine (5), which were difficult to separate by chromatography (Scheme 3). Reaction of the *tris*(dimethylamino)phosphorane (4)[81] to (4R)-[N-(Cbz)]-cycloserine (2) (THF at reflux) was more satisfactory and gave the chloro-adduct (3) in 60% yield (Scheme 4).

Triphenylphosphine and carbon tetrachloride (or bromide) have also been reported as useful reagents for the conversion of an acyl hydrazine to the corresponding acyl hydrazonyl halide.[82] However, treatment of (4R)--[N-(Cbz)]-cycloserine (2) with both of these reagent combinations resulted only in recovery of starting material. The only accessible route in our hands to 3-chloro-(4R)-[N-(Cbz)amino]-4,5-dihydroisoxazole (3) from (4R)-[N-(Cbz)]--cycloserine (2) is by the reaction of (2) with dichloro*tris*(dimethylamino)-phosphorane (4). Although this is not the reagent of choice, since it is hydroscopic and on hydrolysis liberates hexamethylphosphoric triamide (HMPA), it does however furnish a feasible route to chloride (3) and was used with appropriate precautions being taken.

1.2. Reactions of 3-Chloro-(4R)-[N-(benzyloxycarbonyl) amino]-4,5-Dihydroisoxazole with Simple Amine Nucleophiles

The reactivity of the 3-chloro-(4R)-[N-(Cbz)amino]-4,5-dihydro-isoxazole (3) with amine nucleophiles was examined next. Chloride (3) was treated with various amines (methylamine, piperidine, benzylamine, ammonia and tryptamine) and although successful, displacement of the halide was only observed under forcing conditions (sealed tube, 100°C). The yields of the corresponding amine adducts obtained are expressed in Table 1.

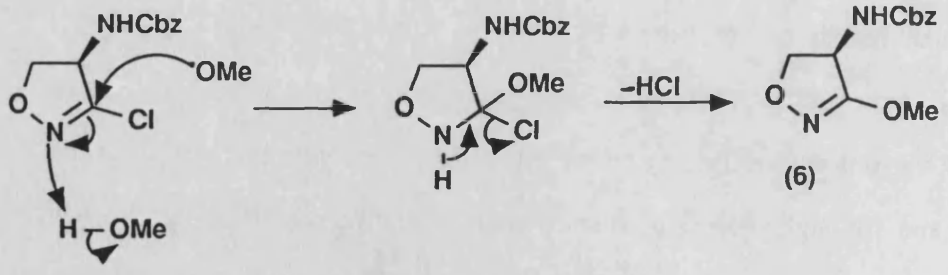
It is apparent that an equilibrium or alternative pathways are established on heating chloride (3) with tryptamine, as varying the reaction time from 48 to 24

Table 2

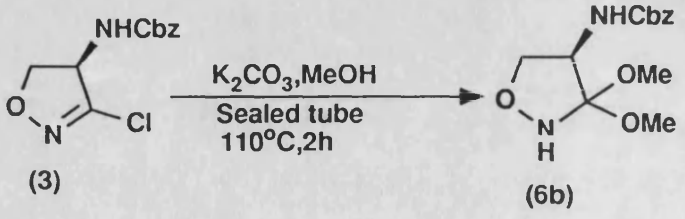
Nucleophile	Base	Solvent	Temp/°C	Rxn Time/h	Results /%
Xs.Gly-OMe.HCl	Xs.K_2CO_3	MeOH	115	2	(6) 26%
—	Xs.K_2CO_3	MeOH	110	2	(6b) 89%
Xs.Gly-OMe	—	Toluene	100	6	(3) 80%
Xs.Gly-OMe.HCl	Et_3N	THF	100-120	48	(3) 88%
(L)-Phe-OMe.HCl	K_2CO_3	DMF	130-160	2	(3)* +complex mixture
(L)-Phe-OMe	—	DMF	130	48	(3)* +complex mixture
(L)-Phe-OMe	—	MeOH	100	48	(3)* +complex mixture
(S)-Leucinol	—	DMF	110	5 days	(3)*
(S)-Leucinol	—	MeOH	110	4 days	(11) 10% (3) 45%

* identified by t.l.c
Xs. =excess

Scheme 5



Scheme 6



hours did not alter the yield of product obtained. Encouraged by our ability to incorporate simple amine nucleophiles into the dihydroisoxazole ring, direct displacement of chloride (**3**) with dipeptide (L)-alanine-7-methyltryptamine was examined. Heating a methanol solution of this dipeptide and (**3**) in a sealed tube at 100°C for 7 days, afforded 3-methoxy derivative (**6**) (25% yield) and recovered starting material (**3**) in 45% yield. Although chloride (**3**) did not couple with the dipeptide (L)-alanine-7-methyltryptamine, we did investigate the displacement of the chloride (**3**) with other amino esters.

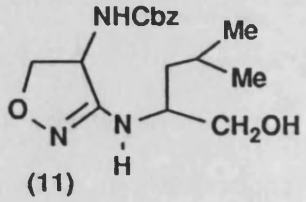
1.1.3. Reactions of 3-Chloro-(4R)-[N-(benzyloxycarbonyl) amino]-4,5-Dihydroisoxazole with α-Amino Esters

All attempts to achieve displacement of chloride (**3**) with nucleophiles derived from amino esters, under the same experimental conditions used earlier for the simple amine nucleophiles, were unsuccessful. The results are expressed in Table 2 and reflect the deactivation of nitrogen by the adjacent ester function in the amino ester. The methoxy adduct (**6**) was isolated upon treatment of chloride (**3**) with glycine methyl ester hydrochloride in the presence of potassium carbonate in methanol. It was proposed that methoxide, generated by the base present, competes with the glycine methyl ester to displace the chloride by an addition-elimination pathway (Scheme 5). To test this hypothesis, chloride (**3**) was subjected to the same experimental conditions as before but in the absence of glycine methyl ester hydrochloride. ^{1}H NMR suggested that the dimethoxy adduct (**6b**) (2x 3H singlet at 3.60 and 3.78ppm) was formed (Scheme 6). This type of methoxylation has been reported previously when 4-substituted-3-chloro--2-isoxazoline was treated with potassium carbonate in methanol.[93c]

Table 3

Nucleophile	anhyd. NaI	Solvent	Temp/°C	Rxn Time/°C	Results /%
(S)-Leucinol	1.1	MeOH	110	3 days	(3) 24 (6) 62 (11) 9
(S)-Leucinol	5	MeOH	110	1 day	loss (3)*
(S)-Leucinol+(6)	15	MeOH	115	2 days	complex mixture
——	5	MeOH	125	1 days	(3)*
(L)-Phe-OMe	5	MeOH	125	2 days	(3) 42% + complex mixture
(S)-Leucinol	1.1	DMF	110	8 days	(3)* + complex mixture
(S)-Leucinol	5	DMSO	120	2 days	decomp.
(S)-Leucinol	5	Acetone	120-130	3 days	complex mixture
(s)-Leucinol	5	CH_3NO_2	120	3 days	(3) 80%

* refers to t.l.c.



(11)

1.1.4. Reactions of 3-Chloro-(4R)-[N-(benzyloxycarbonyl) amino]-4,5-Dihydroxisoxazole with 2-Amino Alcohols

As 2-amino alcohols are better nucleophiles than amino esters, they were used in an attempt to displace the chlorine from (**3**). We envisaged that the alcohol could be oxidised to give the required acid if the displacement was successful. In the event, heating chloride (**3**) with (S)-leucinol in methanol (sealed tube at 110°C for 4 days) gave (S)-leucinol adduct (**11**), but only in 10% yield. In addition, starting material (**3**) and the 3-methoxy adduct (**6**) were isolated in 24% and 62% yields respectively.

We anticipated that addition of sodium iodide would catalyse the above reaction. The corresponding iodo derivative should be more reactive than the chloride (**3**) and displacement by amino alcohol may be promoted. However, treatment of (S)-leucinol with (**3**) in the presence of sodium iodide (1.1 mol equivalent) gave no improvement in isolated yield of the (S)-leucinol adduct (**11**), although reaction was somewhat faster (3 rather than 4 days). Attempts to either detect or isolate the iodo-intermediate failed. Various solvent systems, such as dimethylformamide, dimethylsulphoxide, tetrahydrofuran, acetone and nitromethane with (S)-leucinol and chloride (**3**) in the presence of anhydrous sodium iodide were examined, but none of these gave the (S)-leucinol adduct (**11**). The results of this aspect of the study are summarised in Table 3.

High yields of the 3-methoxy adduct (**6**) were isolated from the (S)-leucinol reaction with chloride (**3**). It is possible that this species may be an/the active intermediate. Subjecting (**6**), under the same reaction conditions as used with chloride (**3**), to (S)-leucinol and sodium iodide only gave a complex mixture and none of the desired adduct was observed.

Table 4

Nucleophile	1M $AgNO_3$	Solvent	Temp/^oC	Rxn Time/days	Results
(S)-Leucinol	0.5 ml	MeOH	100-120	3	(3) 99%
(L)-Phe-OMe	0.5 ml	MeOH	85-100	3	(3) 80%

Scheme 7

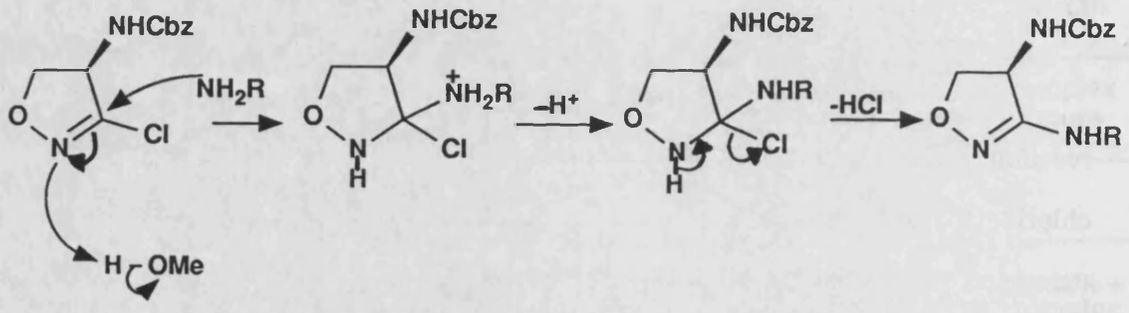


Table 5

Nucleophile	$NH_4^+CH_3CO_2^-$	Solvent	Temp/^oC	Rxn Time/days	Results
Xs.(S)-Leucinol	3 equiv.	MeOH	100	6	(3)
1eq.Benzylamine	1 equiv.	MeOH	100	2.5	(3) 10% (9) 64%

Xs = excess

Silver(I) ions are known to complex strongly to chlorides and we speculated that the addition of a Ag^I salt to chloride (3) might provide the required activation and enhance the rate of the nucleophilic displacement reaction. However, heating a mixture of (3) and either (S)-leucinol or (L)-phenylalanine methyl ester in methanol (in a sealed tube) in the presence of an excess of 1M aqueous silver nitrate solution gave no reaction and starting material was recovered (Table 4).

1.1.5. Addition of a Weak Acid to 3-Chloro-(4R)-[N-(benzyloxycarbonyl)-amino]-4,5-Dihydroisoxazole with 2-Amino Alcohols

It would appear from our results that methanol is the only solvent in which displacement of the chloride (3) will occur (see Tables 2 and 3). This may be because methanol is a weak acid and is therefore acting as the proton source required for the addition-elimination mechanism (Scheme 7). The concomitant formation of methoxide ions would also account for the 3-methoxy adduct (6) which was observed as a by-product in a large number of simple amine displacement reactions of (3) (Table 1).

The addition of a weak acid, ammonium acetate, was then examined (Table 5). Heating (3) with (S)-leucinol in methanol in the presence of three equivalents of ammonium acetate (sealed tube at 100°C for six days) gave only starting material (3) (by TLC). Repeating the above reaction with benzylamine (1 mol equivalent) as the nucleophilic component, gave no significant acceleration (the reaction went to completion in 2.5 days as compared with the original 3 days that had been required).

Table 6

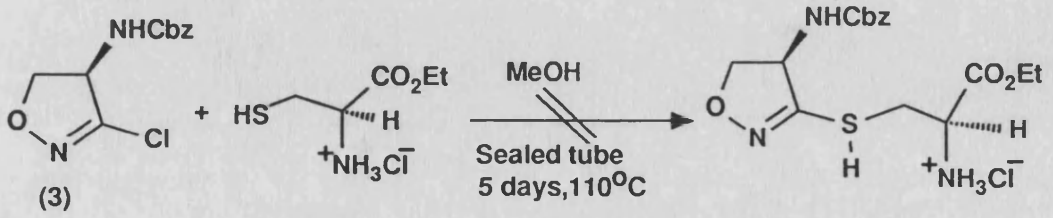
Nucleophile	Solvent	Temp/°C	Rxn Time/h	Results
2.2eq.(L)-Phe$^{-n}Bu_4N^+$	MeOH	100	20	(3) 12% (6) 73%
2.2eq.(L)-Phe$^{-n}Bu_4N^+$	DMF	80	14	complex mixture

1.1.6. Reactions of 3-Chloro-(4R)-[N-(benzyloxycarbonyl)amino]-4,5-Dihydroisoxazole with Carboxylate Salts of α-Amino Acids

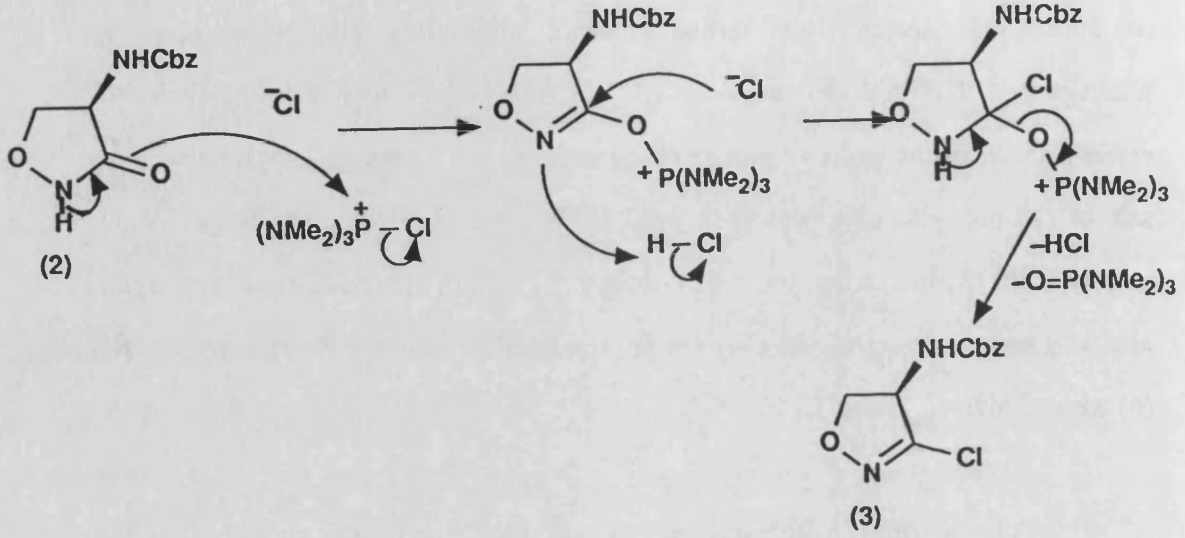
Due to our lack of success in incorporating 2-amino alcohols or α-amino esters to the dihydroisoxazole ring in synthetically useful yields either by direct displacement or *via* activation of (3) with anhydrous sodium iodide, silver nitrate or ammonium acetate, we turned towards alternative and more reactive nucleophiles. The salts of α-amino acids should be better nucleophiles than amino esters because of the negative charge on the carboxyl. The tetra-n-butylammonium salt of (L)-phenylalanine was then used in an attempt to displace the chloride moiety from (3) but under standard conditions, none of the corresponding amino acid adduct was detected and only starting material (3) and the 3-methoxy adduct (6) were recovered (Table 6).

The inertness displayed by chloride (3) towards the more important amine nucleophiles prompted us to briefly examine sulphur-based nucleophiles. Cysteine is one of two primary amino acids that contain a sulphur atom.[83] Treating chloride (3) with (L)-cysteine ethyl ester hydrochloride in methanol (sealed tube, 100°C for five days) afforded only recovered starting material (3) (Scheme 8). This reaction was not investigated further and we are unable to explain for the lack of reactivity observed for the above reaction.

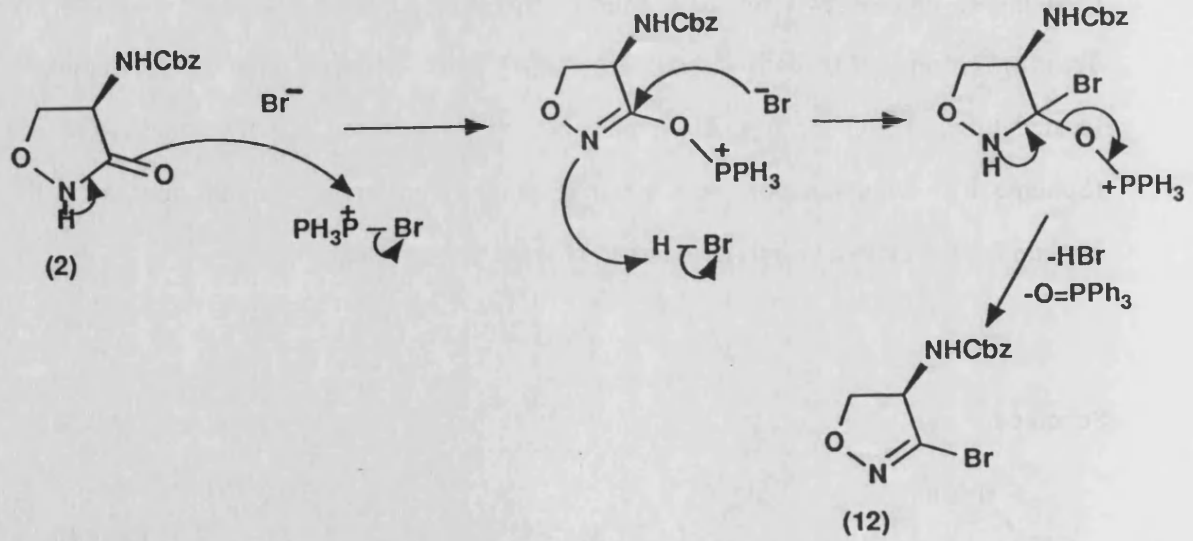
Scheme 8



Scheme 9



Scheme 9b



1.2. Synthesis and Reactivity of 3-Bromo-(4R)-Amino-4,5--Dihydroisoxazole

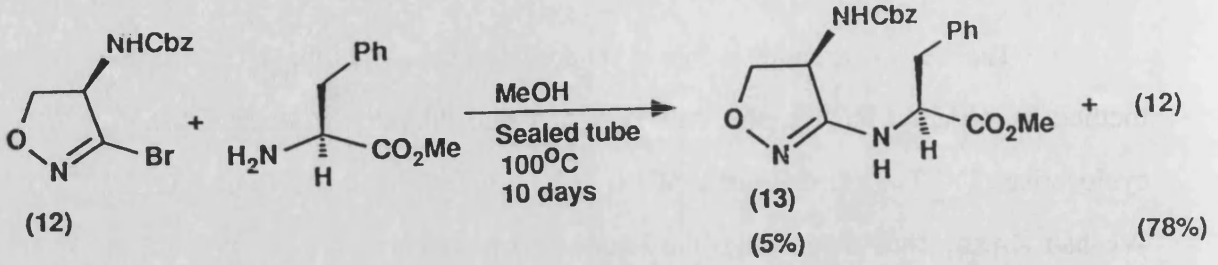
1.2.1. Preparation of 3-Bromo-(4R)-[N-(benzyloxycarbonyl)amino]-4,5-Dihydroisoxazole

The use of a more reactive imidoyl halide would be an alternative method by which to incorporate α-amino esters into the dihydroisoxazole ring of cycloserine (2). The general order of leaving group ability is $I^- > Br^- > Cl^-$.[84] We had already tried to generate the iodide *in situ* but we chose to prepare the imidoyl bromide (12). We did not have any evidence that the iodo derivative had actually formed in the reactions described above. Indeed, the lack of an increase in reactivity when NaI had been added to reactions involving (3) suggested that either the corresponding iodide had not formed, or that the nature of the leaving group was actually unimportant.

We proposed to generate the imidoyl-bromide of (4R)-[N-(Cbz)]--cycloserine (2), by reacting (2) with triphenylphosphine dibromide, a reagent that is commonly employed to convert alcohols to bromides.[85] We speculated that the formation of the desired imidoyl bromide would take place by a mechanism similar to that employed to generate the chloro adduct (3).

The implementation of this task is shown in Schemes 9 and 9b. Treatment of (2) with triphenylphosphine dibromide in THF for 1h at room temperature gave 3-bromo-(4R)-[N-(Cbz)amino]-4,5-dihydroisoxazole (12) in 16% yield and attempts to improve the efficiency of this reaction failed. It is possible that this low yield was due to the presence of hydrogen bromide generated as a by-product during the course of the reaction but when repeated in the presence of 1,2-epoxypropane as an acid scavenger, no trace of the desired product was

Scheme 10


NHCbz
Br
O
N
(12)
+
Ph
H_2N
CO_2Me
H
MeOH
Sealed tube
100°C
10 days
NHCbz
Ph
O
N
N
H
H
CO_2Me
(13)
(5%)
+
(12)
(78%)

observed.

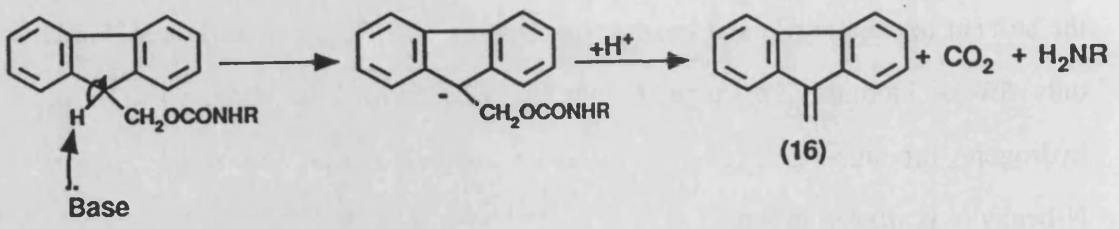
An alternative method of generating a reactive brominating agent is to use triphenylphosphine and carbon tetrabromide.[85] However, reacting (4R)--[N-(Cbz)]-cycloserine (2) with this combination in dichloromethane for five days at 65°C, gave primarily the chloro adduct (3) with < 0.2% of bromine in the compound. This was distinguished by microanalysis and mass spectroscopy and it is apparent that dichloromethane was the origin of the chloride source. Changing the solvent to acetonitrile and heating the mixture at 80°C for five days, afforded only 5% of bromide (12), together with 48% of benzyl bromide. Clearly, the hydrogen bromide generated in this reaction caused cleavage of the N-benzyloxycarbonyl group.

1.2.2. Reactivity of 3-Bromo-(4R)-[N-(benzyloxycarbonyl)amino]-4,5-Dihydroisoxazole with (L)-Phenylalanine Methyl Ester

The reactivity of the bromide (12) towards (L)-phenylalanine methyl ester was then investigated. Treating a methanol solution of bromide (12) with (L)-phenylalanine methyl ester (sealed tube at 100°C for 10 days) afforded recovered starting material (12) (78%), along with 5% of the desired (L)-phenylalanine methyl ester adduct (13) (Scheme 10).

This was our first insight into the incorporation of an α-amino ester into the dihydroisoxazole ring and the bromide-based route appears to be the most promising. The main problem encountered was associated with the incompatibility of the N-protecting group for (4R)-cycloserine, which needs to be stable under acidic conditions required for the synthesis of bromides related to (12).

Scheme 11



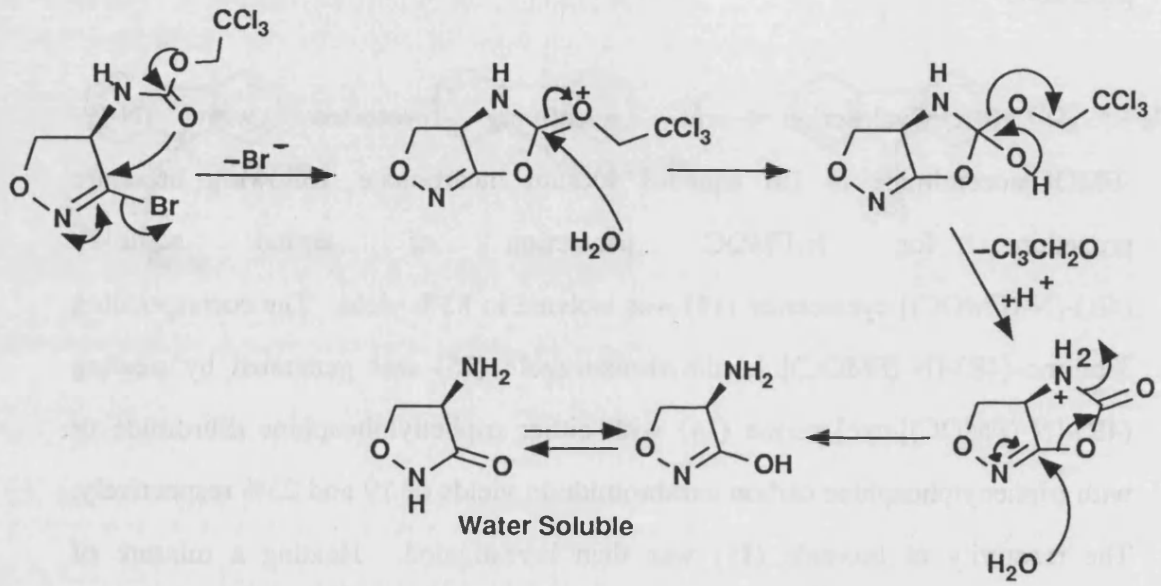
1.2.3. Synthesis and Reactivity of 3-Bromo-(4R)-[N-(9--fluorenylmethoxycarbonyl)amino]4,5-Dihydroisoxazole

9-Fluorenylmethoxycarbonyl (FMOC) has been reported to be an acid stable amine protecting group suitable for α-amino acids and is readily removed under mild alkaline and nonhydrolytic conditions, such as liquid ammonia or neat piperidine.[86,87]

(4R)-Cycloserine was selectively protected with N-(9--FMOC)succinimide in 1M aqueous sodium bicarbonate, following literature procedure for N-FMOC protection of amino acids.[86] (4R)-[N-(FMOC)]-cycloserine (**14**) was isolated in 83% yield. The corresponding 3-bromo-(4R)-[N-(FMOC)]-4,5-dihydroisoxazole (**15**) was generated by treating (4R)-[N-(FMOC)]-cycloserine (**14**) with either triphenylphosphine dibromide or with triphenylphosphine carbon tetrabromide in yields of 19 and 23% respectively. The reactivity of bromide (**15**) was then investigated. Heating a mixture of tryptamine and (**15**) in methanol (sealed tube, 80°C, 4.5 hours) afforded dibenzoflulvene (**16**). Dibenzoflulvene was still observed (by TLC) even when the reaction was conducted at room temperature. Similar results were also observed on treating bromide (**15**) with dipeptide (L)-alanine-7-methyltryptamine (room temperature, 1 hour).

It is evident that both tryptamine and (L)-alanine-7-methyltryptamine are basic enough to remove the acidic proton on the β-carbon of the ethyl carbamate incorporated into the FMOC residue and fragmentation is driven by the aromaticity of the dibenzocyclopentadienyl anion (Scheme 11). There is some literature precedent for the removal of the FMOC protecting group by primary and secondary amines but these reactions usually take place at a much slower rate.[87,88]

Scheme 12


CCl3
H
N
O
O
O
N
Br
−Br−
H
N
+
O
CCl3
O
N
O
H2O
H
N
O
CCl3
O
N
O
O
H
−Cl3CH2O
+H+
H2
N
+
O
O
O
N
H2O
NH2
O
N
H
O
Water Soluble
NH2
O
N
OH

1.2.4. Synthesis and Reactivity of 3-Bromo-(4R)-[N-(2,2,2--trichloroethyloxycarbonyl)amino]-4,5-Dihydroisoxazole and 3-Bromo-(4R)-[N-(4-methylphenyl)sulphonamido]-4,5--Dihydroisoxazole

The incompatibility of FMOC as a protecting group for the amino function of (R)-cycloserine under our reaction conditions led us to examine the the 2,2,2-trichloroethyloxycarbonyl (TROC) group as an alternative. The TROC group has been reported to be stable to both acids and bases[89] but is readily removed reductively by, for example, zinc powder in the presence of acetic acid or methanol.[90,91]

(4R)-[N-(TROC)]-Cycloserine (**17**) (61%) was prepared by treating (R)--cycloserine with 2,2,2-trichloroethyloxy chloroformate. [N-(TROC)]cycloserine (**17**) was then subjected to the usual bromination conditions, however, upon treatment of (**17**) with triphenylphosphine dibromide in dibromomethane or with triphenylphosphine and carbon tetrabromide, none of the desired bromo adduct was detected. Similarly, reacting (**17**) with the more reactive *tris*(dimethylamino)-phosphine dibromide in THF was also unsuccessful.

Our inability to achieve the synthesis of 3-bromo-(4R)-[N-(TROC)-amino]-4,5-dihydroisoxazole (**23**) or to efficiently recover the starting material made us suspect that the carbamate group itself might be participating in the reaction as illustrated in Scheme 12. We proposed that the carbamate group is interacting internally with the imidoyl bromide resulting in the loss of bromide and eventually, the loss of the carbamate protecting group to give cycloserine which is water soluble. The sulphonamide group was then sought to N-protect (R)-cycloserine due to its reknowned stability as an amino protecting group[89b]

displacement reaction.

(R)-Cycloserine was N-protected to give (4R)-[N-(4-methylphenyl)-sulphonyl]-cycloserine (**18**) in 78% yield, using 4-methylphenylsulphonyl chloride under Schotten-Baumann conditions. The use of pyridine as solvent for this reaction gave a complex mixture. Sulphonamide (**18**) was then subjected to the usual brominating agents (triphenylphosphine dibromide or triphenylphosphine with carbon tetrabromide) in a wide variety of solvent systems (THF, dibromomethane, and dimethoxyethane) but, once again, none of the desired bromo adduct was isolated nor could starting material be recovered. TLC scale experiments did indicate that, under the conditions used for work up (1M aqueous sodium bicarbonate or pH 7 buffer), material was being lost into the aqueous phase. Repeating the above reactions and quenching with saturated aqueous ammonium chloride solution (pH 6) gave only recovered starting material, contaminated with triphenylphosphine oxide which results from the hydrolysis of the brominating agent.

Returning to the synthesis of AT-125 from tricholomic acid, it was reported by Kelly *et al*,[79] that exposure of α-[N-(phthalimido)]-3-chloro-4,5-dihydro-5-isoxazole ethyl diphenylmethyl ester (**19**) to hydrogen bromide in nitromethane for <5min at 25°C was not only insufficient to cleave the diphenylmethyl ester, but under these conditions halogen exchange occurred to afford (**20**) as shown in Scheme 13.

Scheme 13

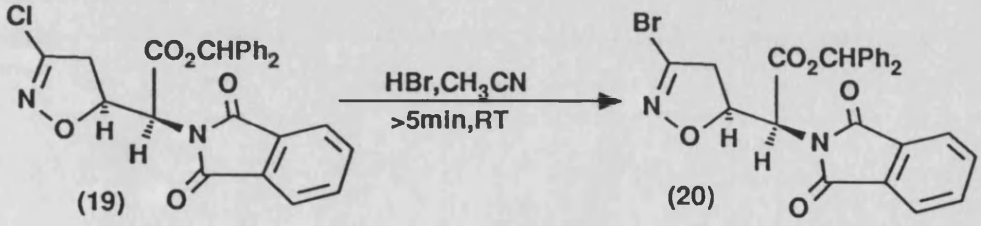
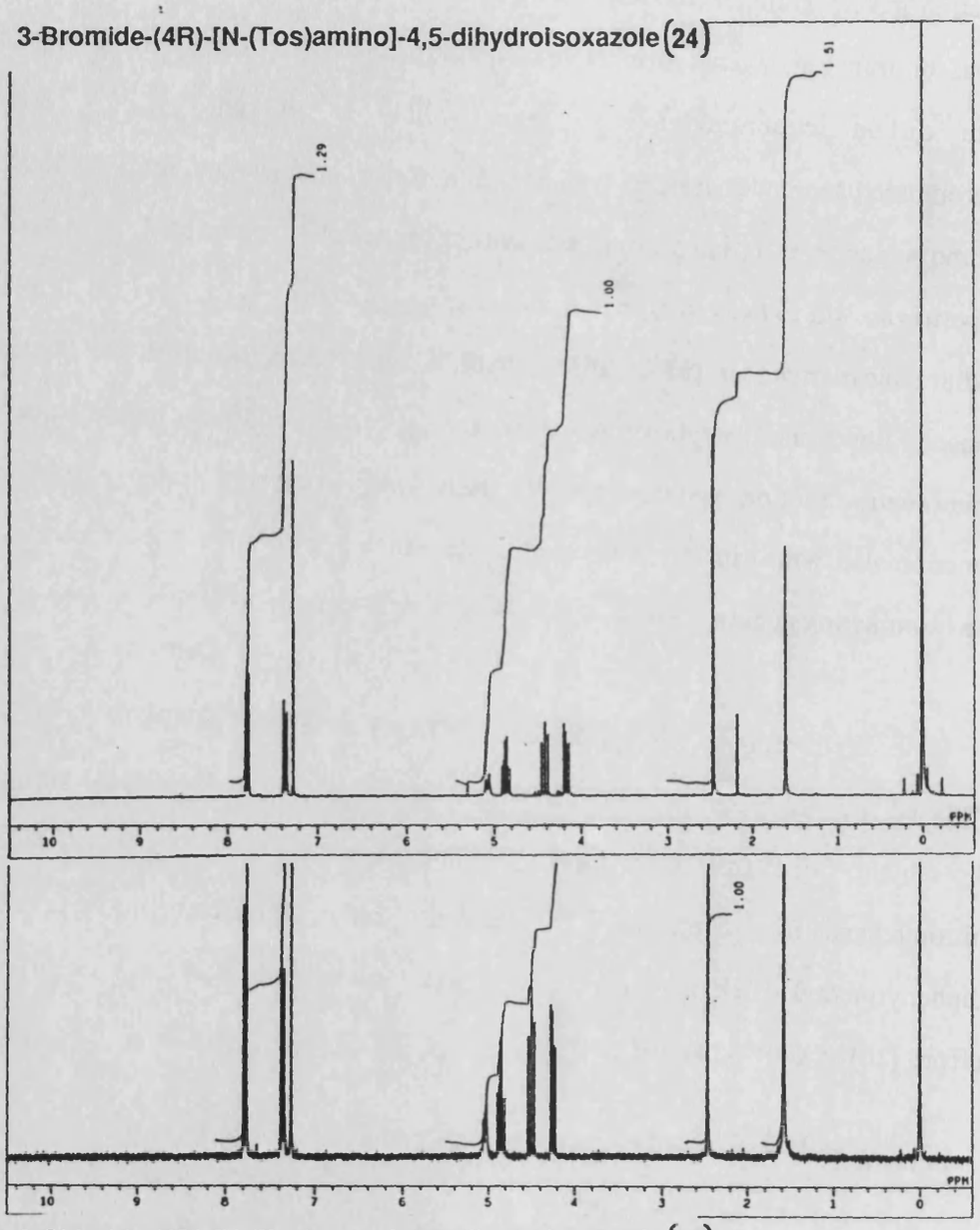


Fig. 1

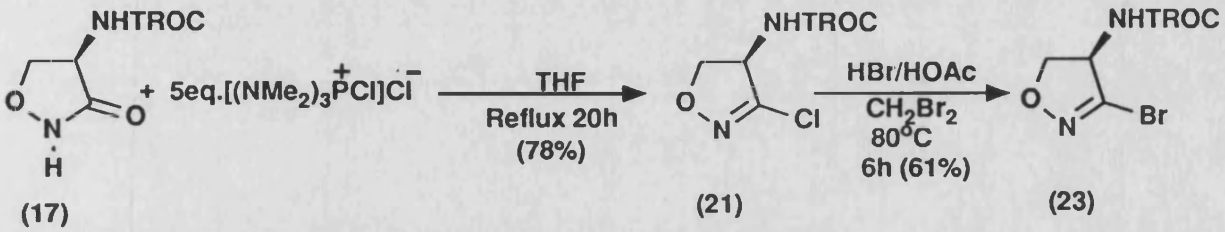
3-Bromide-(4R)-[N-(Tos)amino]-4,5-dihydroisoxazole (24)



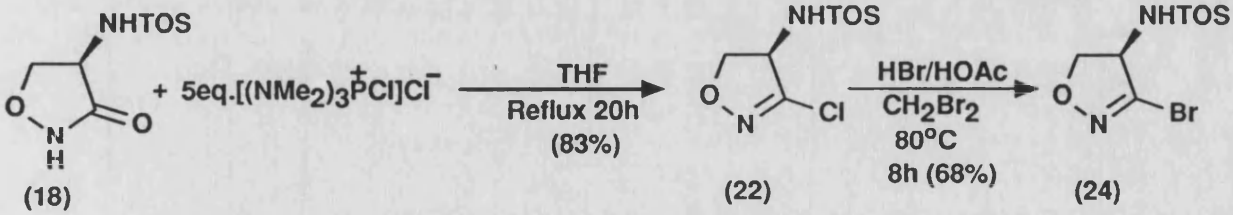
3-Chloride-(4R)-[N-(Tos)amino]-4,5-dihydroisoxazole (22)

We sought to apply this type of exchange process to the synthesis of our key intermediates. As a result, the imidoyl chlorides of (4R)-[N-(TROC)]--cycloserine and (4R)-[N-(Tos)]-cycloserine were both prepared using the same experimental conditions as those used for the preparation of 3-chloro--(4R)-[N-(Cbz)amino]-4,5-dihydroisoxazole (**3**), and were obtained in yields of 78 and 83% respectively. Chlorides (**21**) and (**22**) were then converted to the corresponding imidoyl bromides (**23**) and (**24**) respectively using with 1.1 equivalents of hydrogen bromide in acetic acid (33% wt) (Schemes 14 and 15).

Scheme 14



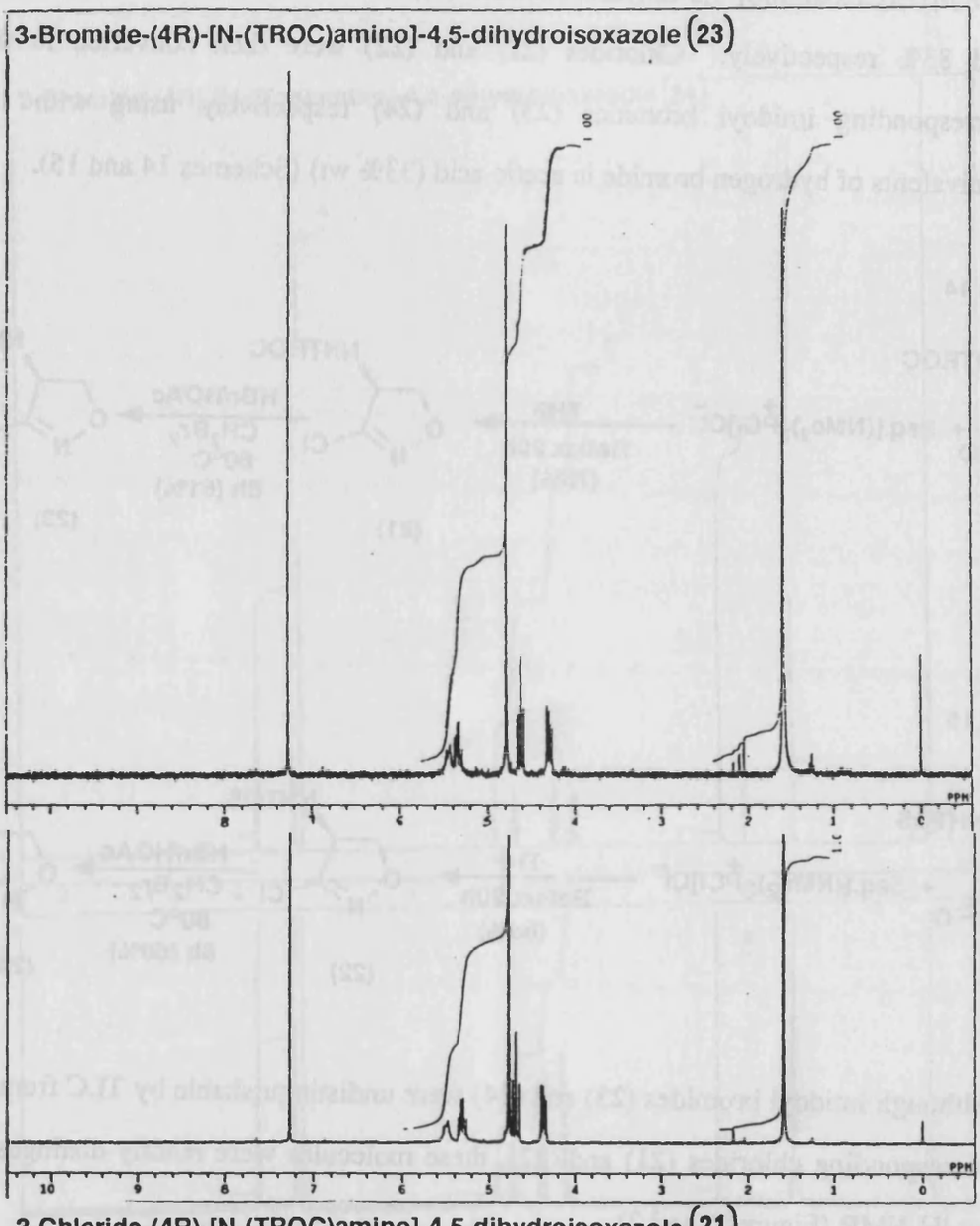
Scheme 15



Although imidoyl bromides (**23**) and (**24**) were undistinguishable by TLC from the corresponding chlorides (**21**) and (**22**), these molecules were readily distingushed by ^{1}H NMR (Figures 1 and 2).

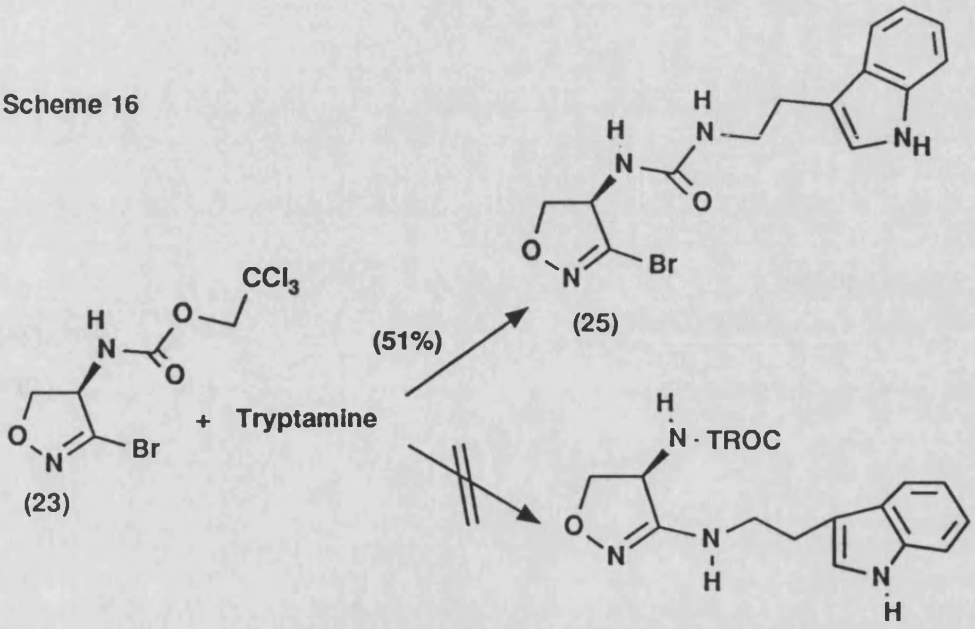
The reactivity of 3-bromo-(4R)-[N-(TROC)amino]-4,5-dihydroisoxazole (**23**) towards tryptamine was then examined. Heating bromide (**23**) with tryptamine in methanol, in a sealed tube at 100°C for 1.5h led to the loss of starting

Fig. 2


3-Bromide-(4R)-[N-(TROC)amino]-4,5-dihydroisoxazole (23)
PPM
3-Chloride-(4R)-[N-(TROC)amino]-4,5-dihydroisoxazole (21)
PPM

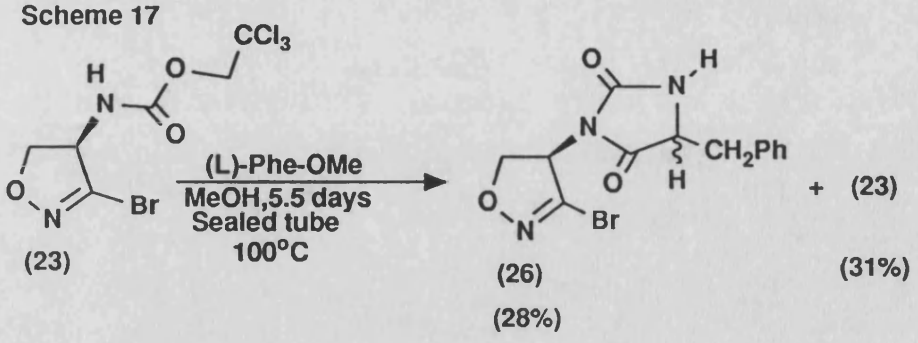
material (**23**) and gave a product (**25**) that contained both the tryptamine unit and a bromine atom, as verified by ^1H NMR and mass spectroscopy (Scheme 16). The structural assignment is based on spectroscopic and microanalytical data obtained.

Scheme 16

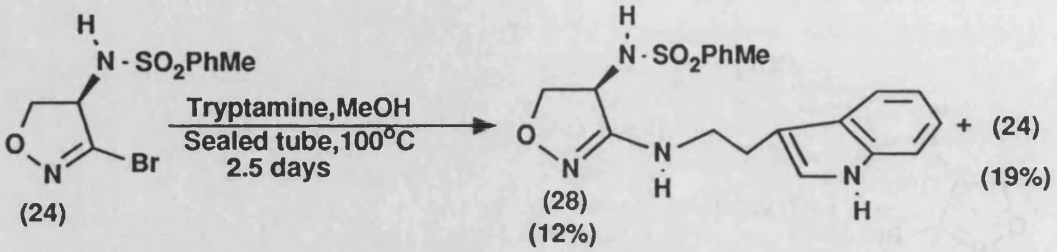


Similarly, reaction of bromide (**23**) with (L)-phenylalanine methyl ester as well as with glycine methyl ester (sealed tube, 100°C) gave imidazolidine--dinone adducts (**26**) and (**27**), where the bromine in the dihydroisoxazole ring is still intact (Scheme 17 and 18).

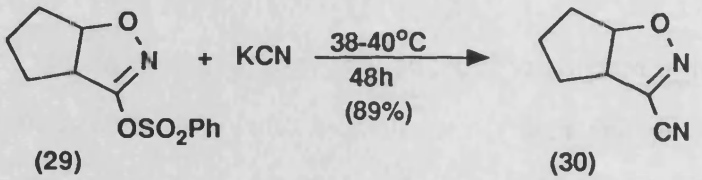
Scheme 17

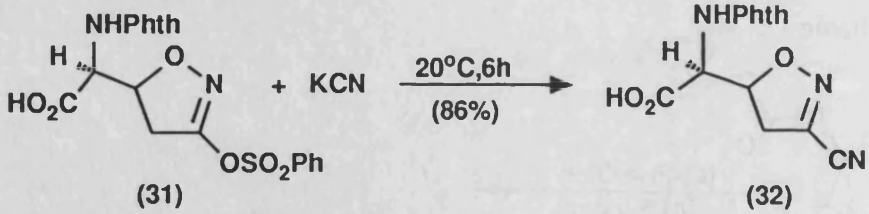


Scheme 19

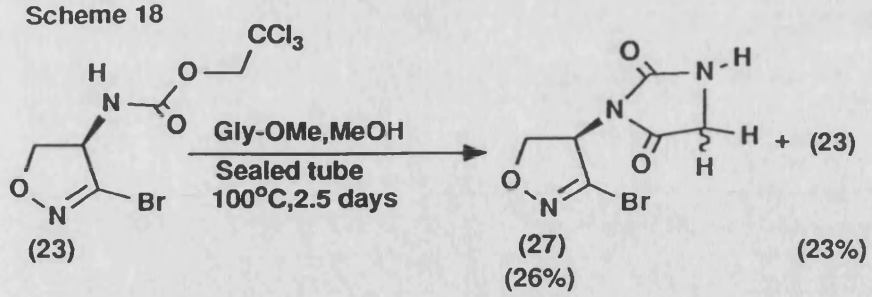


Scheme 20





Scheme 18

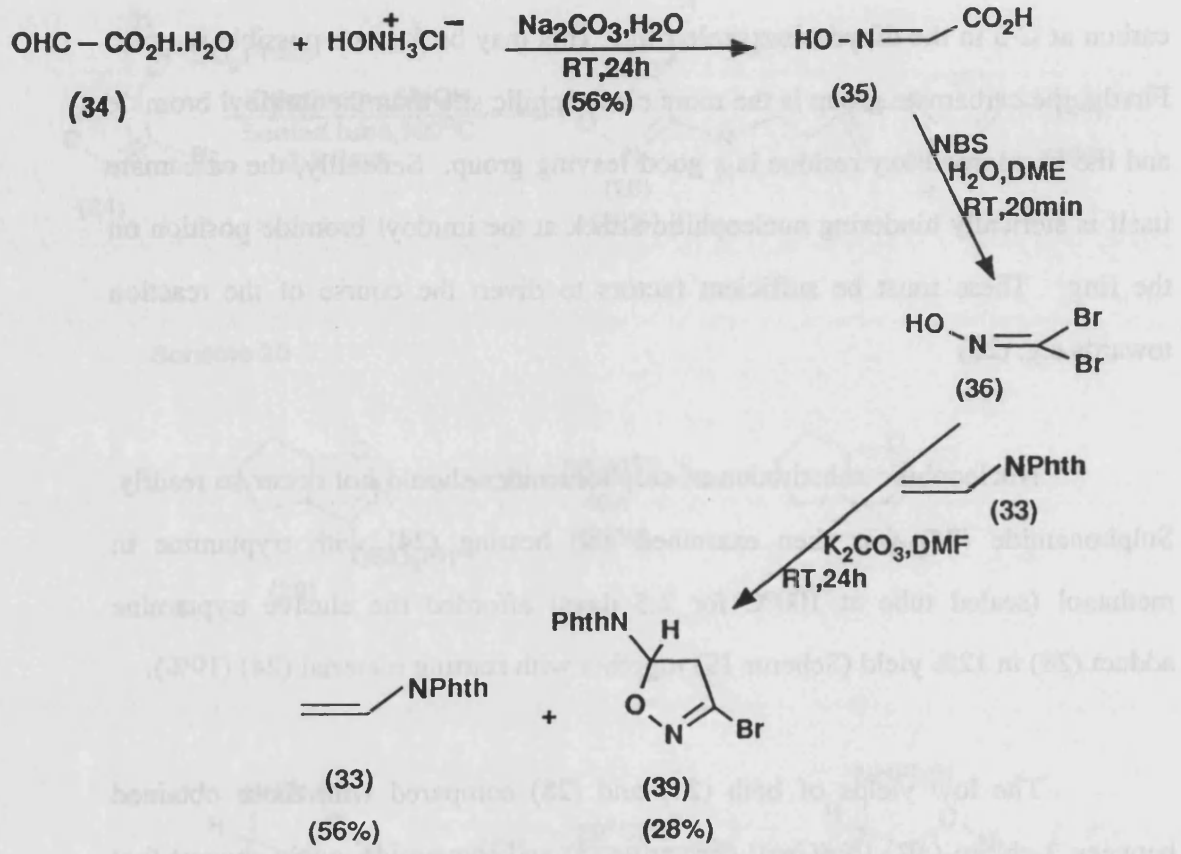


It is apparent that the trichloroethyloxycarbonyl group is more suspectible to nucleophilic attack by amines and amino esters than the imine carbon at C-3 in the dihydroisoxazole ring. This may be for two possible reasons. Firstly, the carbamate group is the more electrophilic site than the imidoyl bromide and the trichloroethoxy residue is a good leaving group. Secondly, the carbamate itself is sterically hindering nucleophilic attack at the imidoyl bromide position on the ring. These must be sufficient factors to divert the course of the reaction towards e.g. (25)

Nucleophilic substitution of sulphonamides should not occur so readily. Sulphonamide (24) was then examined and heating (24) with tryptamine in methanol (sealed tube at 100°C for 2.5 days) afforded the elusive tryptamine adduct (28) in 12% yield (Scheme 19) together with starting material (24) (19%).

The low yields of both (24) and (28) compared with those obtained between 3-chloro-(4R)-[N-(Cbz)] derivative (3) and tryptamine, again suggest that the tosyl protecting group is hindering nucleophilic attack at the imidoyl bromide position. Indeed, this type of substitution was also found to be difficult in 3-O-phenylsulphonyl-isoxazolidine.[92] It was reported that the 4,5-disubstituted 3-O-phenylsulphonyl-isoxazolidine (29) undergoes a much slower reaction with cyanide ions than the corresponding 4-unsubsitituted isoxazolidine (31) (Scheme 20). It is presumed that branching at C-4 of the isoxazolidine ring slows down attack at adjacent C-3 position.

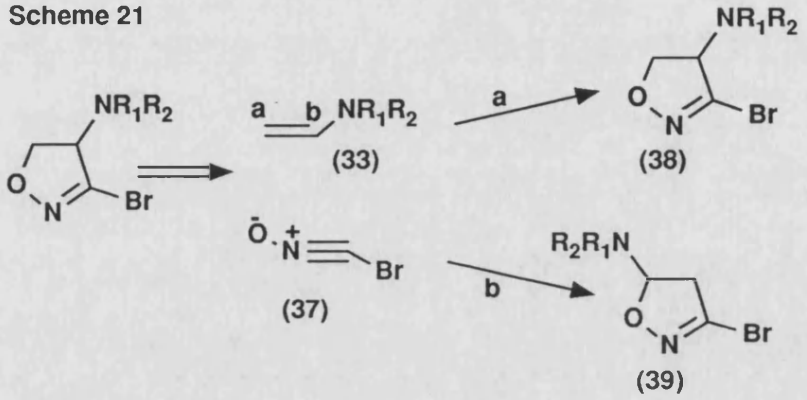
Scheme 22


OHC – CO2H.H2O
(34)
+ HONH3Cl
Na2CO3,H2O
RT,24h
(56%)
HO – N
CO2H
(35)
NBS
H2O,DME
RT,20min
HO
N
Br
Br
(36)
NPhth
(33)
K2CO3,DMF
RT,24h
PhthN
H
O
N
Br
NPhth
(33)
(56%)
(39)
(28%)

1.2.5. Attempt to Synthesize 3-Bromo-4-[N-(phthaloyl)amino]-4,5--Dihydroisoxazole via a 1,3 Dipolar Cyclisation Reaction

An independent route to 3-bromo-4-[N-(phthaloyl)amino]-4,5-dihydro--isoxazole (**38**) was also undertaken. The use of 1,3 dipolar cycloadditions for the synthesis of the 3-substituted dihydroisoxazole ring sustem is well established in the literature.[93a-e] We planned to synthesize (**38**) by cycloaddition of the reactive bromonitrile oxide (**37**) to vinylphthalimide (**33**) as illustrated in Scheme 21.

Scheme 21

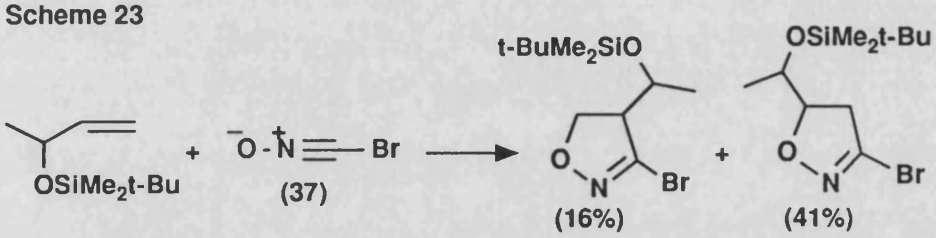


Vinylphthalimide (**33**) was prepared in 64% yield from a reaction of phthalimide with vinyl acetate using sodium tetrachloropalladate as catalyst.[94] Bromonitrile oxide (**37**) was generated *in situ* from dibromoformaldoxime (**36**) by reaction of (**36**) with potassium hydrogen carbonate. Dibromoformaldoxime (**36**) is also best generated *in situ*, due to its high toxicity,[95] and it was derived from dihalogenation of glyoxalic acid aldoxime (**35**) with N-bromosuccimide.[93] Using literature procedure, glyoxalic acid aldoxime (**35**) was prepared, in 56% yield, from glyoxalic acid monohydrate (**34**) and hydroxylamine hydrochloride[96] and the overall reaction sequence is outlined in Scheme 22.

There are two possible regioisomers, 4-[N-(phthaloyl)amino]--dihydroisoxazole (**38**) and 5-[N-(phthaloyl)amino]-dihydroisoxazole (**39**), that can arise from this cycloaddition. It has been reported that 1-substituted alkenes react

with bromonitrile oxide to give predominantly or exclusively the 5-substituted dihydroisoxazole.[93e] There is however, one reported incident where a 4-substituted dihydroisoxazole was derived, due to the presence of the bulky 3-(*tert*-butyldimethylsilyloxy) group on the dipolarophile[93e] (Scheme 23), and for this reason, a bulky phthaloyl was used as the protecting group for the vinylamine and cycloaddition attempted.

Scheme 23



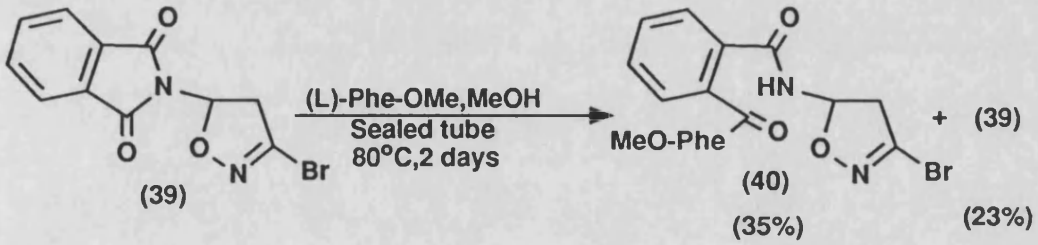
In the event, reaction of vinylphthalimide with bromonitrile oxide gave exclusively the 5-[N-(phthaloyl)amino]-dihydroisoxazole (**39**) (28%), along with starting material (**33**) (56%). Regioselectivity in 1,3-dipolar cycloaddition can be explained by the frontier molecular orbital theory.[138] 1-Substituted dipolarophiles have been reported to have the largest coefficient at the β-carbon regardless whether the substituent is conjugated, electron-withdrawing or electron-donating or in either of the frontier orbitals (HOMO or LUMO). 1,3-Dipolar cycloaddition are therefore dipole-HOMO or dipole-LUMO controlled. As there are no reported energies for the frontier orbitals or coefficient valves for bromonitrile oxide (the dipole), we are therefore unable to speculate whether the above reaction is dipole-HOMO or dipole-LUMO controlled. We are however, able to conclude that the largest coefficient is on the carbon atom of the bromonitrile oxide, since only 5-[N-(phthaloyl)amino]-dihydroisoxazole (**39**) was formed.

1.2.6. Reactivity of 3-Bromo-5-[N-(phthaloyl)amino]-4,5-Dihydroisoxazole

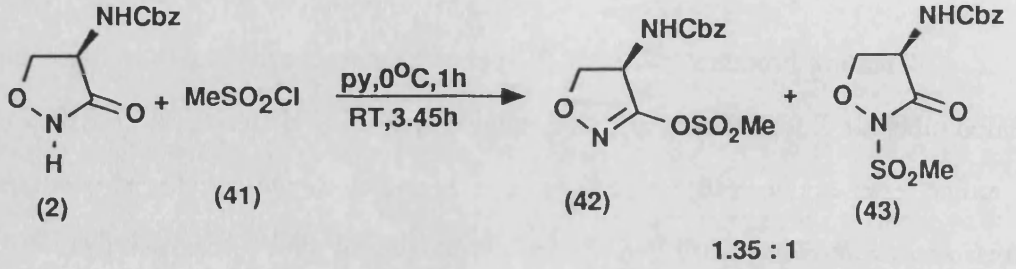
To probe the lack of reactivity of the 3-bromo-4-substituted dihydroisoxazoles with nucleophiles, we next examined the reaction of 3-bromo-5-[N-(phthaloyl)amino]-4,5-dihydroisoxazole (**39**) with α-amino esters. This system represents a sterically-less hindered imidoyl bromide and was of interest in relation to our earlier results with the 4-amino derivatives.

Treating bromide (**39**) with (L)-phenylalanine methyl ester in methanol (sealed tube, 80°C for 2 days), afforded recovered starting material (**39**) (23%) and an amino ester adduct (**40**) in 35% yield, *which still contained bromine in the dihydroisoxazole ring* (confirmed by mass spectroscopy and microanalysis). It is once again evident that the nucleophile reacted preferentially with the phthalimide protecting group rather than with the imidoyl bromide (Scheme 24).

Scheme 24



Scheme 25a



(2) (41) (42) (43)

1.35 : 1

1.3. Synthesis and Reactivity of 3-Methanesulphonyloxy-(4R)-[N-(benzyloxycarbonyl)amino]-4,5-Dihydroisoxazole and 2-N-Methanesulphonyl-(4R)-[N-(benzyloxycarbonyl)amino]--3-Isoxazolidinone

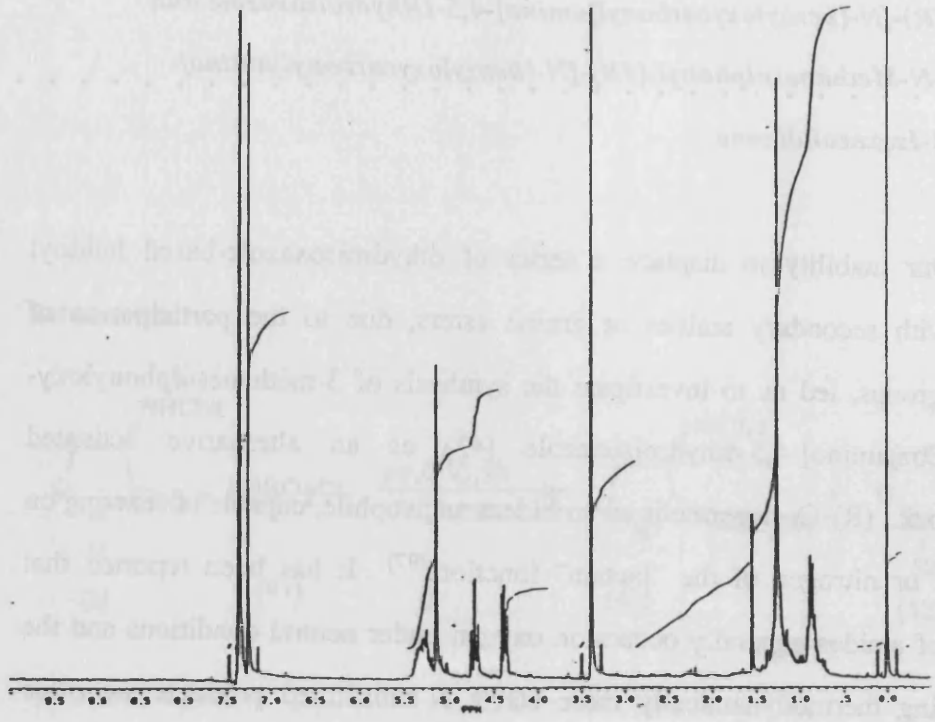
Our inability to displace a series of dihydroisoxazole-based imidoyl bromides with secondary amines or amino esters, due to the participation of protecting groups, led us to investigate the synthesis of 3-methanesulphonyloxy--(4R)-[N-(Cbz)amino]-4,5-dihydroisoxazole (**42**) as an alternative activated building block. (R)-Cycloserine is an ambident nucleophile, capable of reacting on the oxygen or nitrogen of the "lactam" function.[97] It has been reported that alkylation of amides generally occurs on oxygen under neutral conditions and the corresponding thermodynamically more stable N-substituted products are often isolated as a result of rearrangement of the kinetically-formed O-substituted product and this type of rearrangement is generally observed at high temperatures.[98a,b]

With this in mind, the O-mesylation of (4R)-[N-(Cbz)]-cycloserine (**2**) was attempted retaining the N-benzyloxycarbonyl group as it did not appear to participate in reactions involving amines or amino esters (*see section 1.20 and 1.23*). Treatment of (4R)-[N-(Cbz)]-cycloserine (**2**) with methanesulphonyl chloride in pyridine afforded two products as a 1.35:1 mixture (by NMR) corresponding to the O-mesyl dihydroisoxazole (**42**) and the N-mesyl-3--isoxazolidinone (**43**) respectively (Scheme 25a).

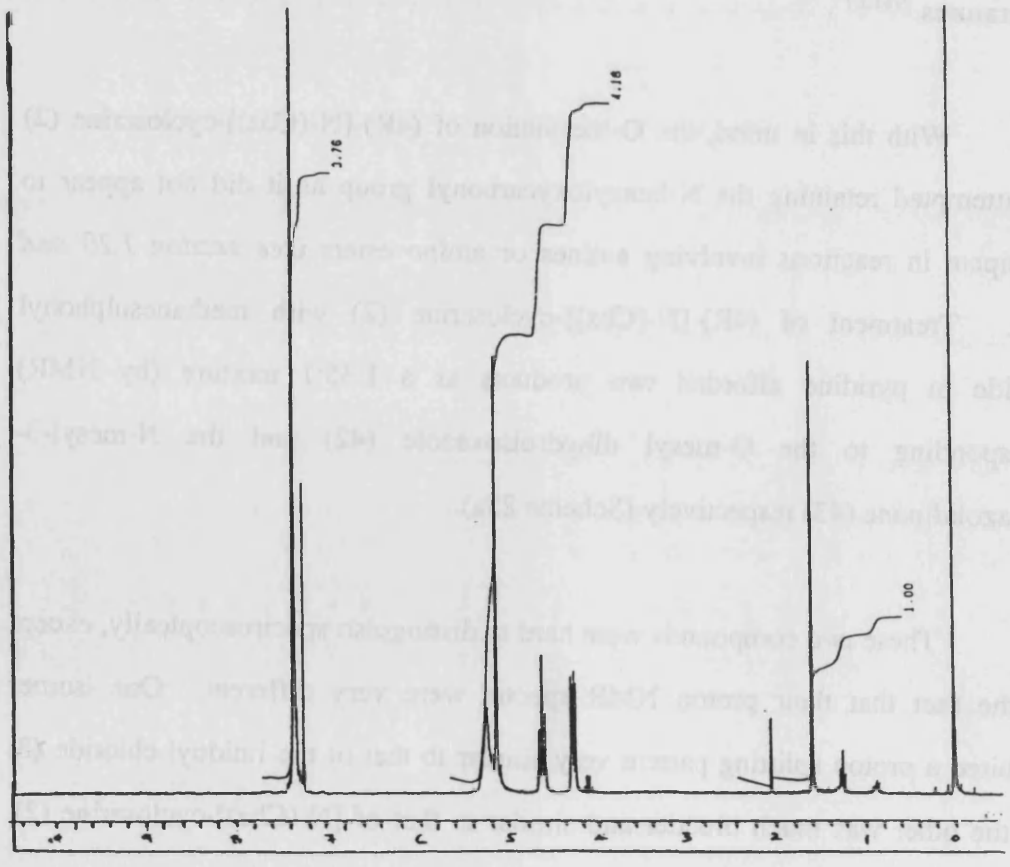
These two compounds were hard to distinguish spectroscopically, except for the fact that their proton NMR spectra were very different. One isomer exhibited a proton splitting pattern very similar to that of the imidoyl chloride (**3**) and the other was much broader and similar to that of [N-(Cbz)]-cycloserine (**2**).

Fig. 3

3-Methanesulphonyloxy-(4R)-[N-(Cbz)amino]-4,5-dihydroisoxazole (42)



3-Chloro-(4R)-[N-(Cbz)amino]-4,5-dihydroisoxazole (3)



Based on these observations the former material was destinated as the O-methanesulphonyl dihydroisoxazole (**42**) and the latter as the N-methanesulphonyl-3-isoxazolidinone isomer (**43**). The relevant ^1H NMR spectra are shown in Figures 3 and 4.

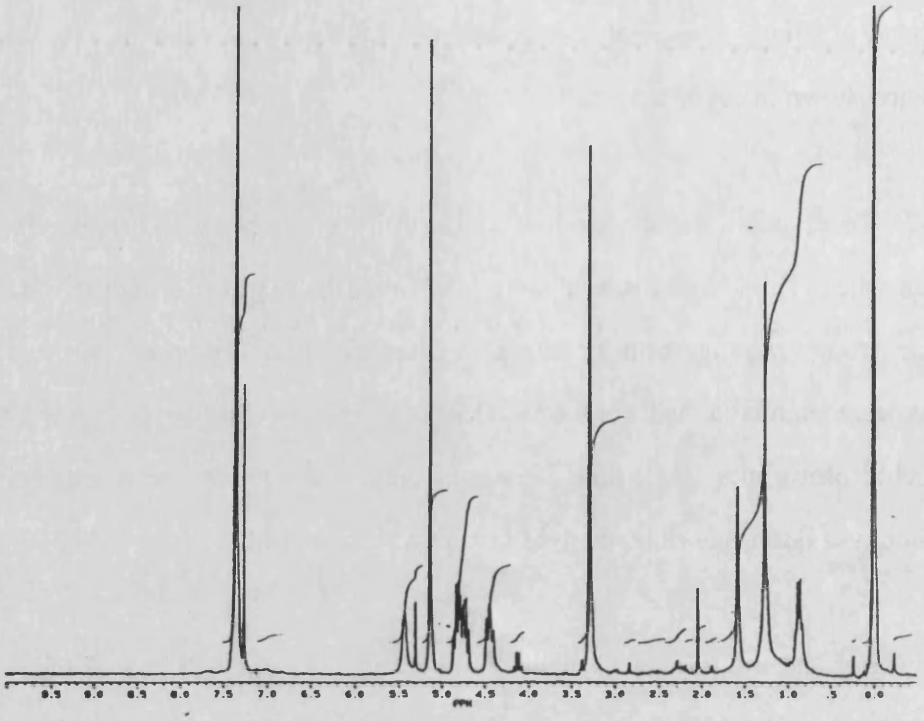
To establish the thermodynamic stability of these two compounds and ascertain whether the N-methanesulphonyl (**43**) was the result of direct mesylation or arose from rearrangement of the O-methanesulphonyl isomer (**42**). Both samples were premixed with one equivalent of (4R)-[N-(Cbz)]-cycloserine (**2**) in deuterochloroform in a NMR tube. These samples were left at room temperature for eight days. After that time, the NMR spectra were recorded.

For the mixture of O-methanesulphonyl (**42**) and (**2**), no change was observed in the ^1H NMR spectra was observed over this time period. However, the mixture of N-methanesulphonyl (**43**) and (**2**), a new peak (at 2.9 ppm) was observed in the ^1H NMR spectrum after two days; no further change was observed after 8 days. We speculated that this new peak is due to methanesulphonic acid (assigned by comparison with the spectrum reproduced in the Aldrich ^1H NMR catalogue[99]) and has arisen from slow hydrolysis of N-methanesulphonyl isomer (**43**). Addition of a small amount of water to the N-methanesulphonyl sample resulted in a substantial increase in intensity of this peak. Hence, we have established that O-methanesulphonyl (**42**) is relatively stable but that the N-methanesulphonyl isomer (**43**) is susceptible to hydrolysis. This experiment also supports that (**43**) is formed from direct mesylation on nitrogen, rather than by rearrangement of (**42**).

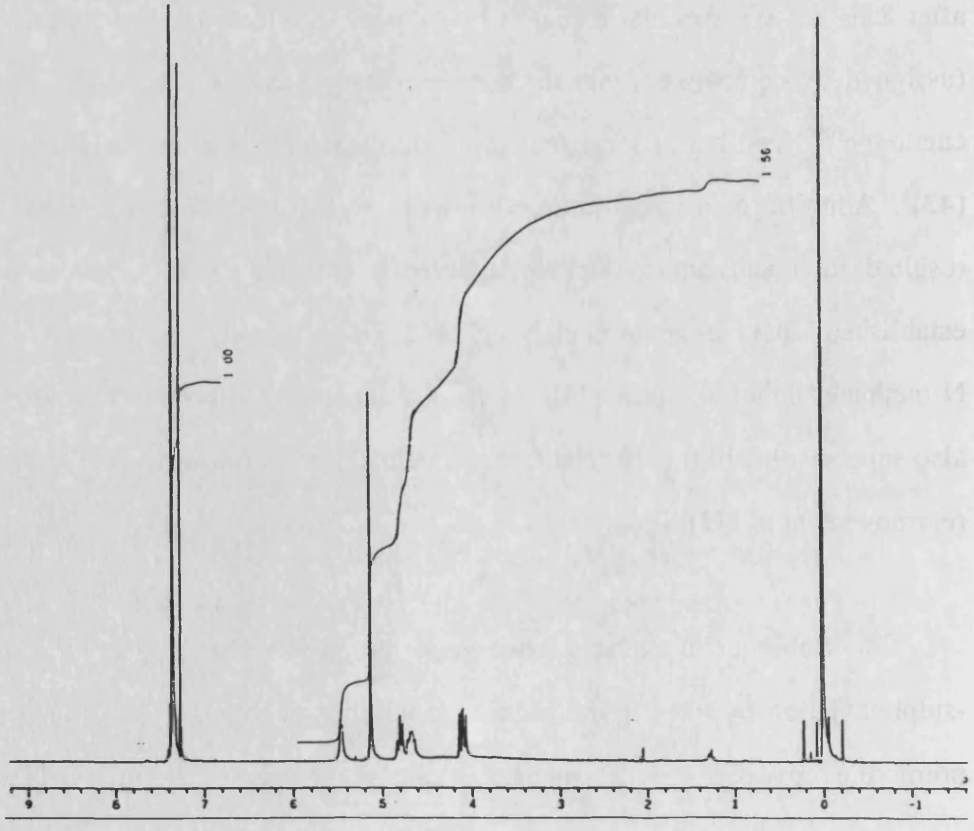
Subsequent attempts were made to improve the ratio of O- vs N--sulphonylation by varying the reaction conditions (Table 7). We found that, on premixing pyridine with methanesulphonyl chloride before addition of

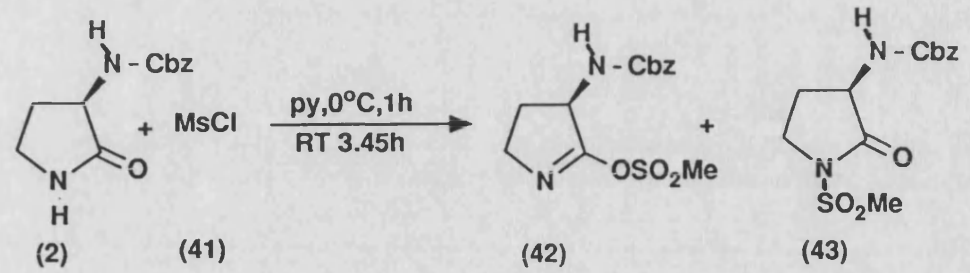
Fig. 4

2-N-Methanesulphonyl-(4R)-[N-(Cbz)amino]-4,5-dihydroisoxazole (43)



(4R)-[N-(Cbz)]-cycloserine (2)





(2) (41) (42) (43)

Table 7

(2)/eq.	(41)/eq.	Base/eq.	Solvent	Rxn conditions	(42) vs (43)
1	1.1	/	py	* (41)+(2) at 0°C, stir 0°C 1h, RT 4h	1.35 : 1
1	10	/	py	* (41)+(2) at 0°C, stir 0°C 1h, RT 3.45h	1.6 : 1
1	1.1	1.1 py	CH_2Cl_2	* (41)+(2) at 0°C, then add py, stir 0°C 1h, RT 11h	1.6 : 1
1	1.1	1.1 Et_3N	CH_2Cl_2	* (41)+(2) at 0°C, then add Et_3N, stir 0°C 0.5h	1 : 2.5
1	1.1	1.1 py	CH_2Cl_2	* (41)+py at 0°C, then add (2), stir 0°C 1h, RT 20h	2 : 1
1	1.1	1.1 py 1.1 DMAP	CH_2Cl_2	* (41)+py+DMAP at 0°C, then add (2), stir 0°C 10 min, RT 1h	4 : 1

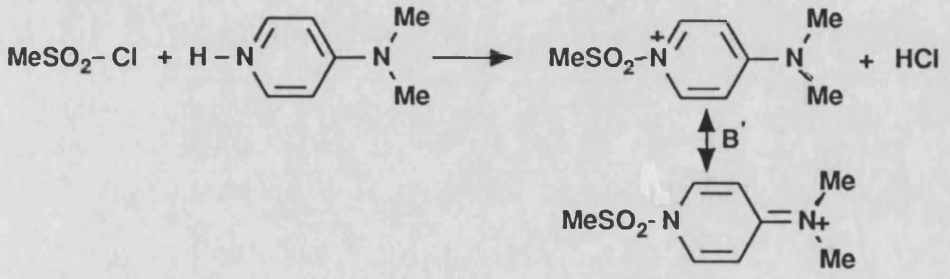
* refer to premixing

[N-(Cbz)]-cycloserine (**2**), that a 2:1 mixture in favour of (**42**) was obtained. It is believed that, under those reaction conditions, a pyridinyl-methanesulphonyl species (A′) is formed which is more reactive than methanesulphonyl chloride, due to the greater leaving group ability associated with the pyridinyl cation[100a] (Scheme 25b).

Scheme 25b

We also sought to speed up the mesylation process by addition of 4-(dimethylamino)pyridine (DMAP) to a CH_2Cl_2 solution of methanesulphonyl chloride and pyridine. DMAP[100b,c] is more nucleophilic than pyridine and will react with methanesulphonyl chloride much quicker to form species (B′) which is stabilised by the dimethylamino residue (Scheme 25c). In this case, a 4:1 mixture was obtained, favouring the desired O-methanesulphonyl isomer (**42**).

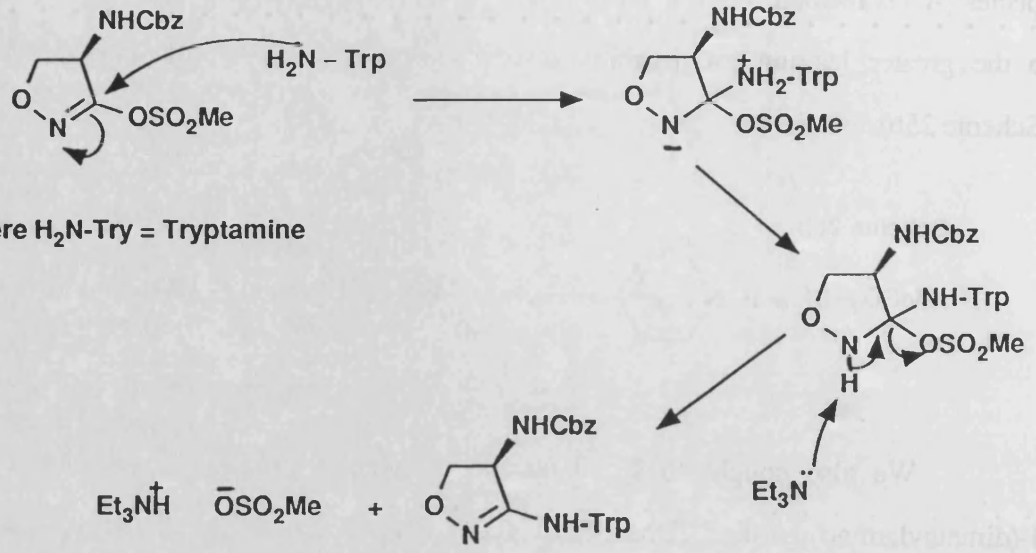
Scheme 25c



As predicted by literature precedent,[98] under strong alkaline conditions, the N-alkylated product was favoured. Treatment of (4R)-[N-(Cbz)]-cycloserine with methanesulphonyl chloride in the presence of triethylamine (pKa 10.7)[101] gave a 1:2 mixture of products favouring the N-methanesulphonyl isomer (**43**).

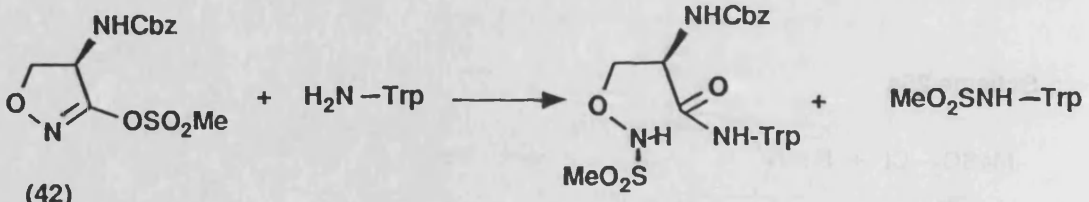
With sufficient quantity of O-methanesulphonyl (**42**) in hand its reactivity towards tryptamine and with (L)-alanine-7-methyltryptamine was

Scheme 26



Where H_2N-Try = Tryptamine

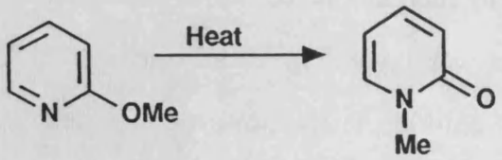
Scheme 26b



(42) (44) (45)

Where H_2N-Try = Tryptamine

Scheme 26c



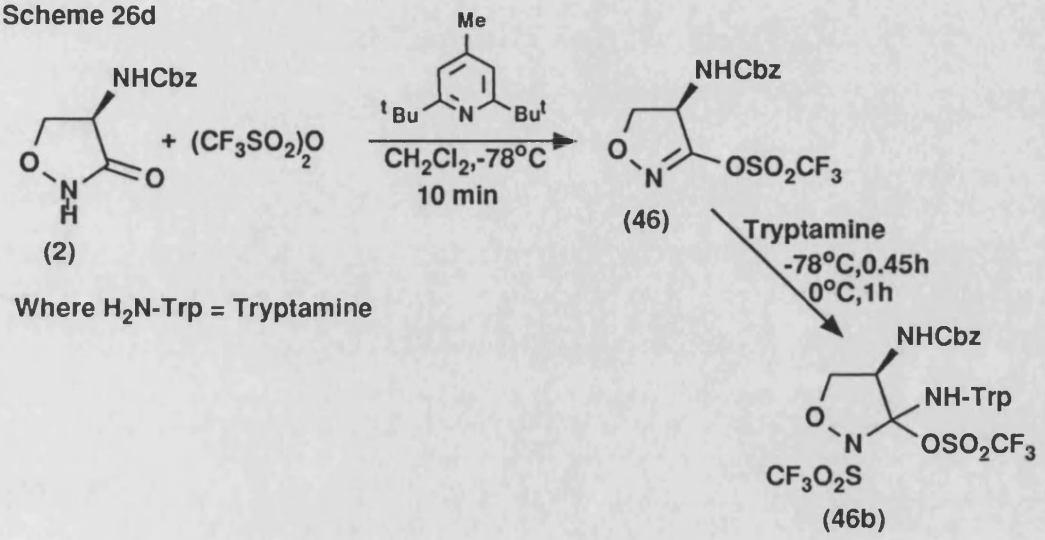
examined. Heating a methanol solution of (**42**) and each amine in turn (50°C for 2 hours) afforded complex mixtures that were not investigated further. Clearly, the O-methanesulphonyl isomer (**42**) is more reactive than the chloro derivative (**3**), which did not react with the dipeptide substrate even though this required heating at 100°C for seven days. Under milder reaction conditions, (**42**) was stirred with tryptamine in methanol at room temperature, in the presence of triethylamine. We envisaged that the addition of base would aid the displacement reaction as shown in Scheme 26. However, the only product isolated corresponded to the ring-opened dihydroisoxazole adduct (**44**), with the methanesulphonyl group now transferred onto the nitrogen in the dihydroisoxazole ring. The structure of (**44**) was assigned by ^{1}H and ^{13}C NMR spectroscopy. 2-N-methanesulphonyl--[3indolylethyl]amine (**45**) was also isolated in 8% yield from this reaction (Scheme 26b). This reaction probably takes place by an intermolecular rather than intramolecular mechanism,[98,102a] which would involve a strained four-membered transition state. This type of 1,3 shift has indeed been reported to occur intermolecularly and may be illustrated by the rearrangement of 2-methoxypyridine to N-methylpyridone (Scheme 26c).[102b] Furthermore, the presence of the N-sulphonyl residue would make nitrogen a better leaving group and thus make the ring-opening a facile process.

The same ring-opened tryptamine adduct (**44**) was also produced by reaction of the N-methanesulphonyl derivative (**43**) with tryptamine, under our standard reaction conditions. It is apparent that the use of O-methanesulphonyl isomer (**42**) as a reactive intermediate for coupling with amino esters is not a productive strategy due to the transfer of the mesyl group from oxygen to nitrogen and, eventually, to ring-opened products.

We have also examined the synthesis of the corresponding 3-trifluoro--methanesulphonyloxy-4,5-dihydroisoxazole (**46**). (4R)-[N-(Cbz)]cycloserine (**2**)

was reacted with triflic anhydride in the presence of 2,6-(di-*tert*-butyl)--4-(methyl)pyridine in dichloromethane at -78°C but upon work up, only a complex mixture of products was obtained. We tried to trap the putative triflate adduct by quenching the reaction mixture with tryptamine (Scheme 26d). Repeating the above reaction in the presence of tryptamine, afforded an adduct (**46b**), which contained tryptamine and two trifluoromethanesulphonyl groups incorporated into the dihydroisoxazole ring. The structure of this product was assigned based on spectroscopic data obtained from ^{1}H, ^{13}C, ^{19}F and ^{1}H-^{1}H 2D COSY experiments.

Scheme 26d

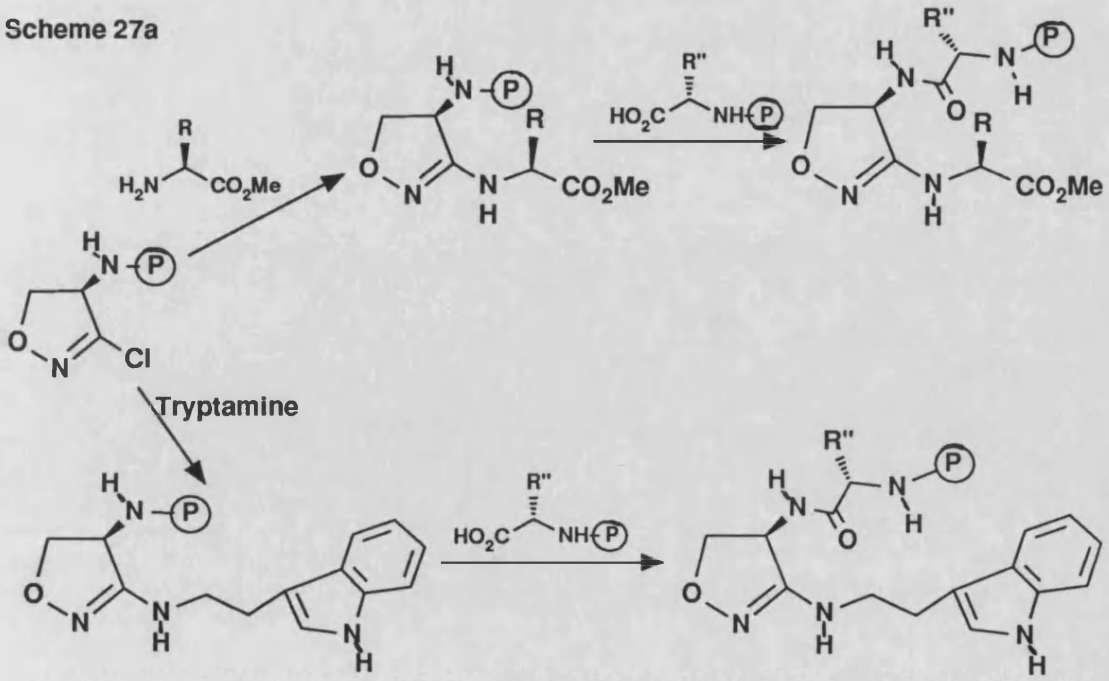


Where H_2N-Trp = Tryptamine

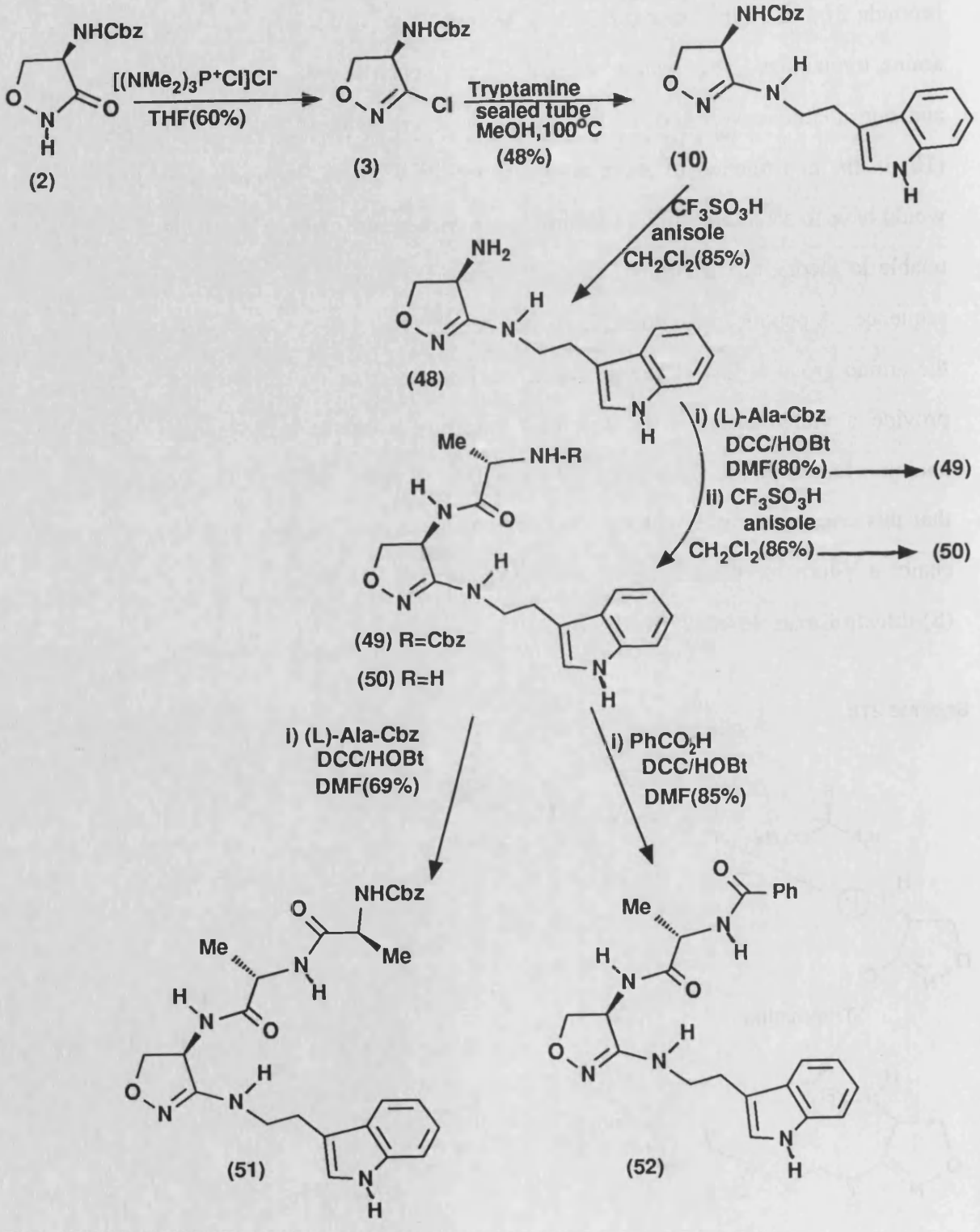
1.4. Synthesis of Tri and Tetrapeptides of 3-Tryptamino-(4R) and (4S)-[N-(Cbz)amino]-4,5-Dihydroisoxazole

Although amino acids could not be used to displace either chloride or bromide from the dihydroisoxazole ring we were able to incorporate the primary amine tryptamine. This residue was of direct interest to Glaxo Group Research and our attentions were focused on the use of 3-tryptamino-4,5-dihydroisoxazole (**10**) in the construction of more complex peptides. In this context, tryptamine would have to act as a terminal function, since without the carboxyl moiety, we are unable to incorporate the dihydroisoxazole mimetic into the middle of a peptide sequence. A peptide chain could, however, be built up by coupling amino acids to the amino group at C-4 of the dihydroisoxazole ring (Scheme 27a). This would provide a viable means with which to construct a constrained peptide chain analogue that could then be studied for possible turn conformations.[103] We hoped that this constraint would enforce one of two turn conformations on the peptide chain; a γ-turn for the (R)-dihydroisoxazole unit and an inverse γ-turn for the (S)-dihydroisoxazole (*see Introduction 2.0*).

Scheme 27a

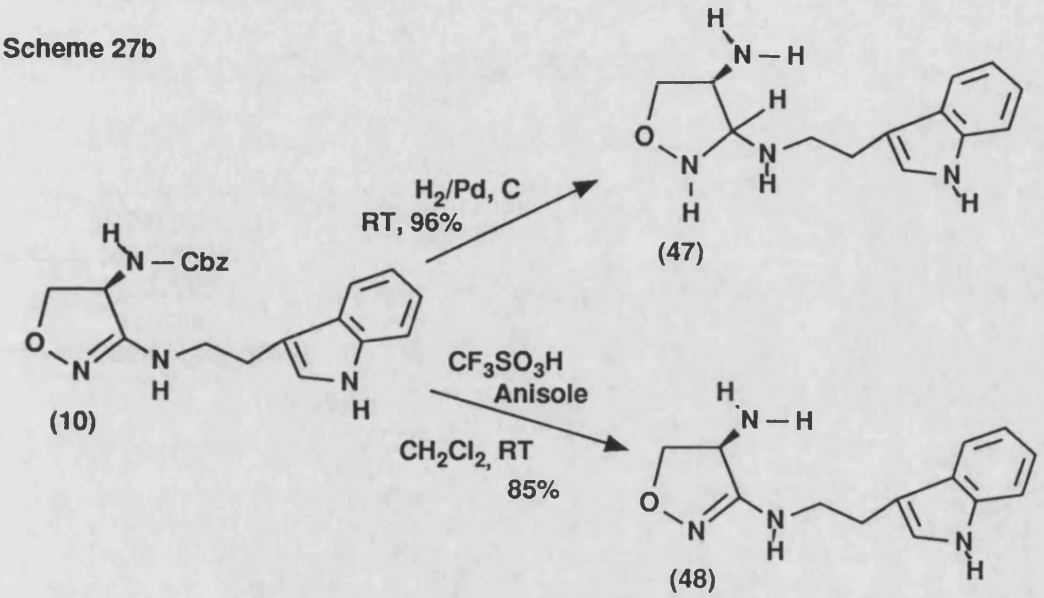


Scheme 28a


NHCbz
[(NMe2)3P+Cl]Cl-
THF(60%)
Cl
Tryptamine
sealed tube
MeOH,100°C
(48%)
(2)
(3)
(10)
CF3SO3H
anisole
CH2Cl2(85%)
NH2
(48)
i) (L)-Ala-Cbz
DCC/HOBt
DMF(80%)
(49)
ii) CF3SO3H
anisole
CH2Cl2(86%)
(50)
Me
NH-R
(49) R=Cbz
(50) R=H
i) (L)-Ala-Cbz
DCC/HOBt
DMF(69%)
i) PhCO2H
DCC/HOBt
DMF(85%)
Ph
(51)
(52)

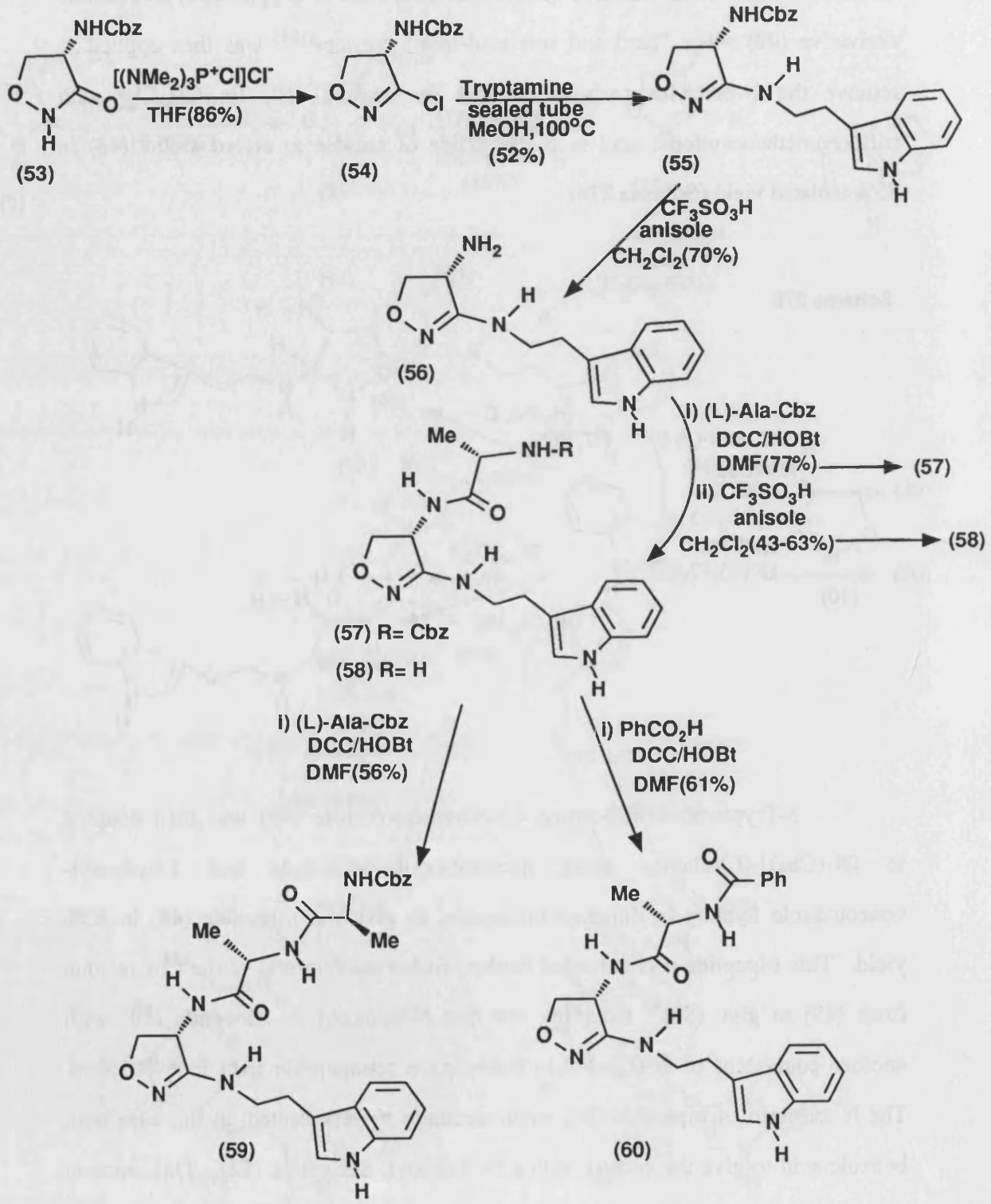
The first step in this sequence required that the N-benzyloxycarbonyl group be removed from the 3-tryptamino derivative (**10**). Although the N-Cbz group is generally removed under catalytic hydrogenation conditions,[89c] in our hands the imine bond was also reduced to afford the 3-(tryptamino)-isoxazoline derivative (**47**). The "hard and soft acid-base" concept[104] was then applied to remove the N-benzyloxycarbonyl at C-4 by treating (**10**) (in CH_2Cl_2) with trifluoromethanesulfonic acid in the presence of anisole to afford amine (**48**) in 85% isolated yield (Scheme 27b).

Scheme 27b



3-Tryptamino-(4R)-amino-4,5-dihydroisoxazole (**48**) was then coupled to [N-(Cbz)]-(L)-alanine using dicyclohexylcarbodiimide and 1-hydroxyl-benzotriazole hydrate in dimethylformamide, to give the tripeptide (**49**) in 80% yield. This tripeptide was extended further, following removal of the Cbz residue from (**49**) to give (**50**). Coupling the free N-terminus of tripeptide (**50**) with another equivalent of N-(Cbz)-(L)-alanine gave tetrapeptide (**51**) in 69% yield. The N-terminus of tripeptide (**50**) could similarly be reprotected, in this case with benzoic acid to give the corresponding N-(benzoyl) derivative (**52**). This reaction was conducted under standard peptide coupling conditions[105] rather than using benzoyl chloride in pyridine as this latter procedure resulted in epimerization. The

Scheme 28b


NHCbz
[(NMe2)3P+Cl]Cl-
THF(86%)
Cl
Tryptamine
sealed tube
MeOH,100°C
(52%)
(53)
(54)
(55)
CF3SO3H
anisole
CH2Cl2(70%)
NH2
(56)
i) (L)-Ala-Cbz
DCC/HOBt
DMF(77%)
(57)
ii) CF3SO3H
anisole
CH2Cl2(43-63%)
(58)
Me
NH-R
(57) R= Cbz
(58) R= H
i) (L)-Ala-Cbz
DCC/HOBt
DMF(56%)
i) PhCO2H
DCC/HOBt
DMF(61%)
Ph
(59)
(60)

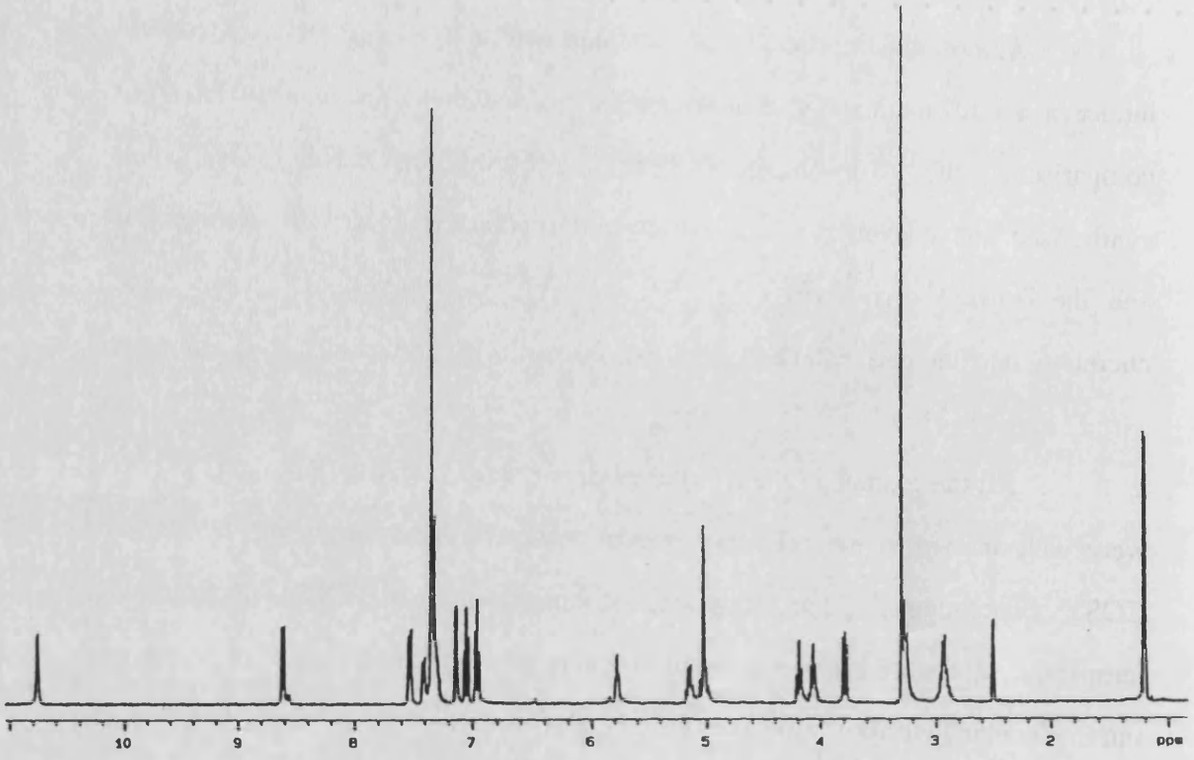
overall synthesis of the tri- and tetrapeptides incorporating the dihydroisoxazole unit is shown in Scheme 28a.

As described earlier, the constrained (4R)-dihydroisoxazole unit should induce a γ-turn and the (S)-enantiomer should induce an inverse γ-turn. For comparison, the 3-tryptamino-(4S)-amino-4,5-dihydroisoxazole (**56**) was synthesised and converted to the corresponding diastereomeric tri-, tetrapeptide and the benzoyl derivatives (**57**), (**59**) and (**60**) respectively, using the same chemistry that had been established for the (R)-series (Scheme 28b).

All the protons in the tri- and tetrapeptide of both the (R)- and (S)-series were well-defined in the ^1H NMR spectra and were assigned using ^1H-^1H 2D COSY experiments. Further spectroscopic studies (ROESY and variable temperature ^1H NMR) on these tri and tetrapeptides, however, failed to detect an intramolecular hydrogen bond between the NH at C-2 with the NH at C-4 of the dihydroisoxazole ring (Figures 5, 6, 7 and 8). This does not rule out the possibility that the peptide chains does not involve a γ or inverse γ-turn, rather we have demonstrated that the peptide chains do not exist exclusively in either a γ or inverse γ-turn conformation on the NMR time scale. Although the solid state conformation may not necessarily be a good model as the solution conformation, we felt that such information would nevertheless be of interest. However, we were unable to obtain satisfactory crystals from any of these derivatives for x-ray crystallographic analysis.

Fig. 5

Tripeptide ; [3′R]-N-[2-[[3-[[2-(3-indolyl)ethyl]amino]-4,5-dihydro-4-isoxazolylamino]-1-methyl-2-oxoethyl]-carbamic acid, phenylmethyl ester (49)



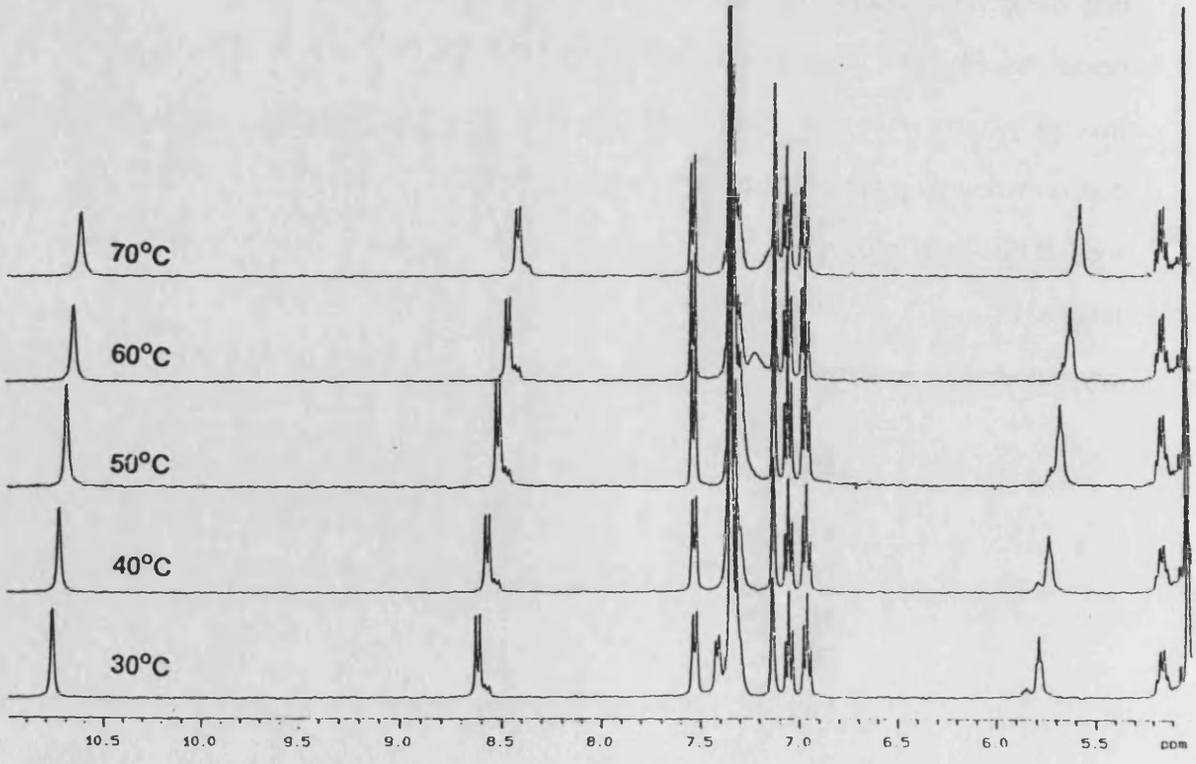
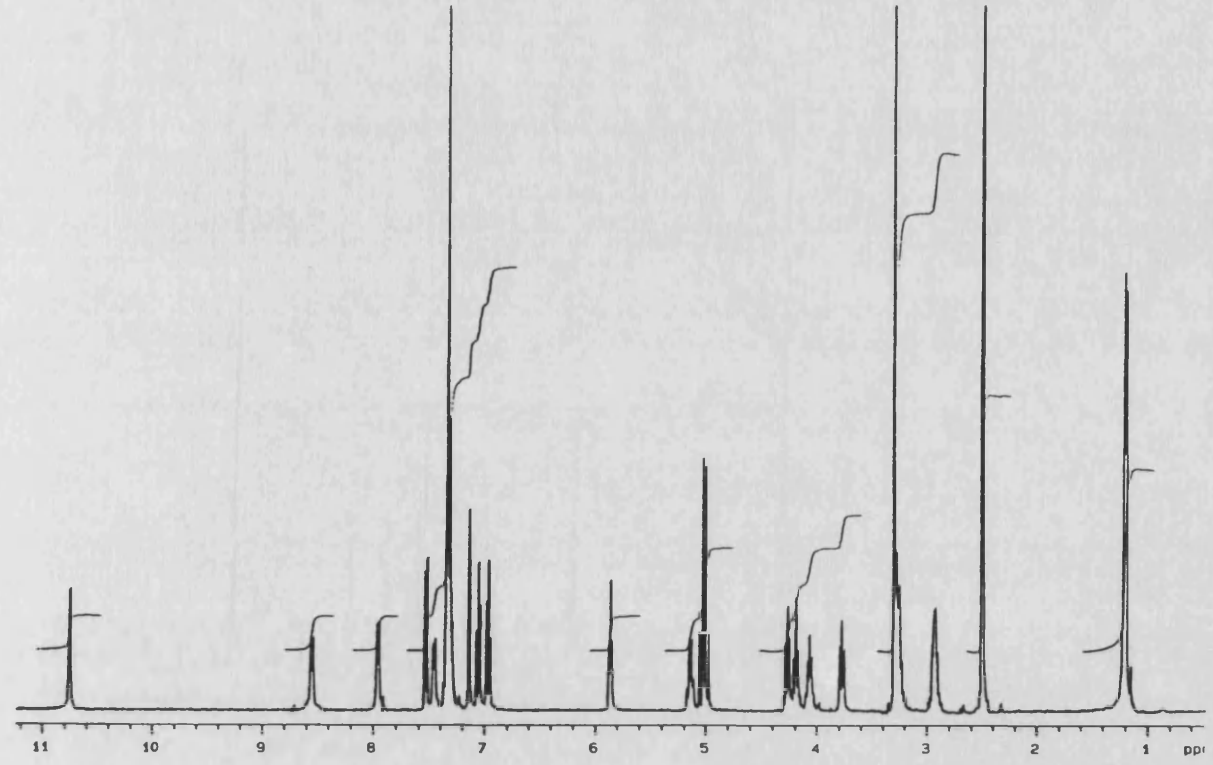


Fig. 6

Tetrapeptide : [3′R]-N-[2-[2-[[3-[[2-(3-indolyl)ethyl]amino]-4,5-dihydro-4-isoxazolylamino]-1-methyl-2-oxoethyl]amino]-1-methyl-2-oxoethyl]carbamic acid, phenylmethyl ester (51)



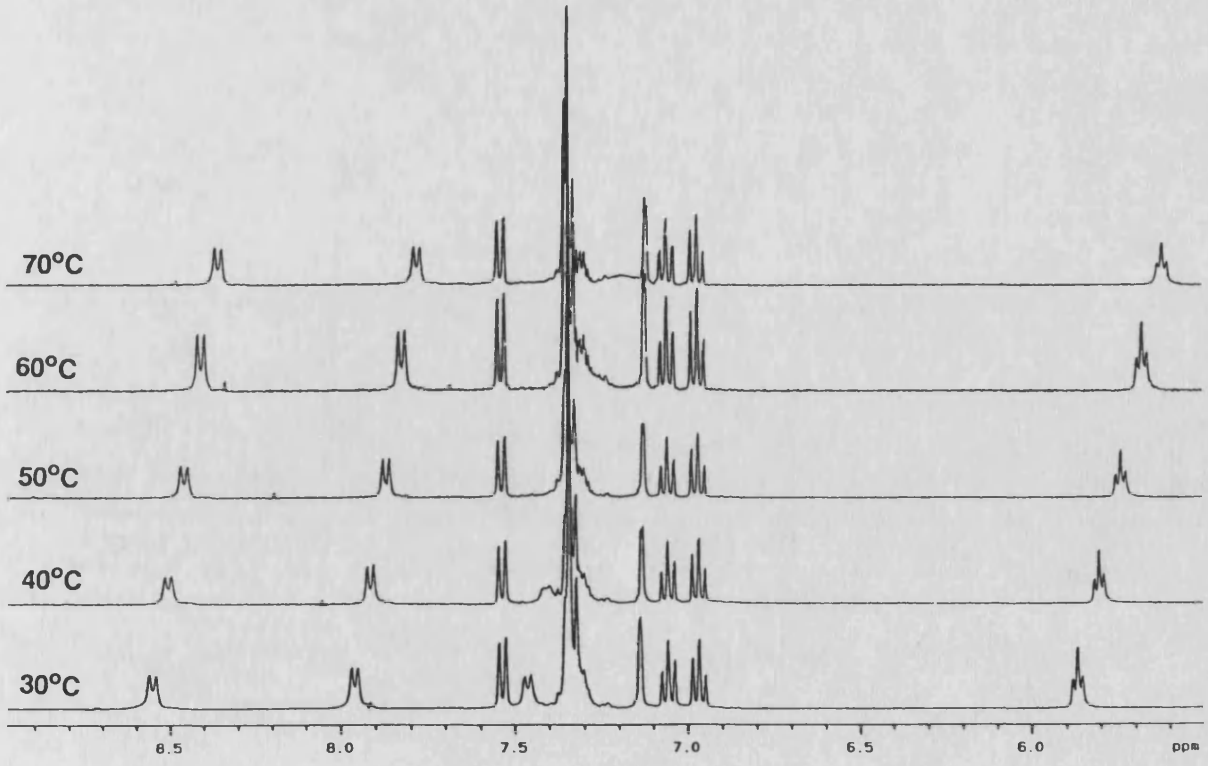
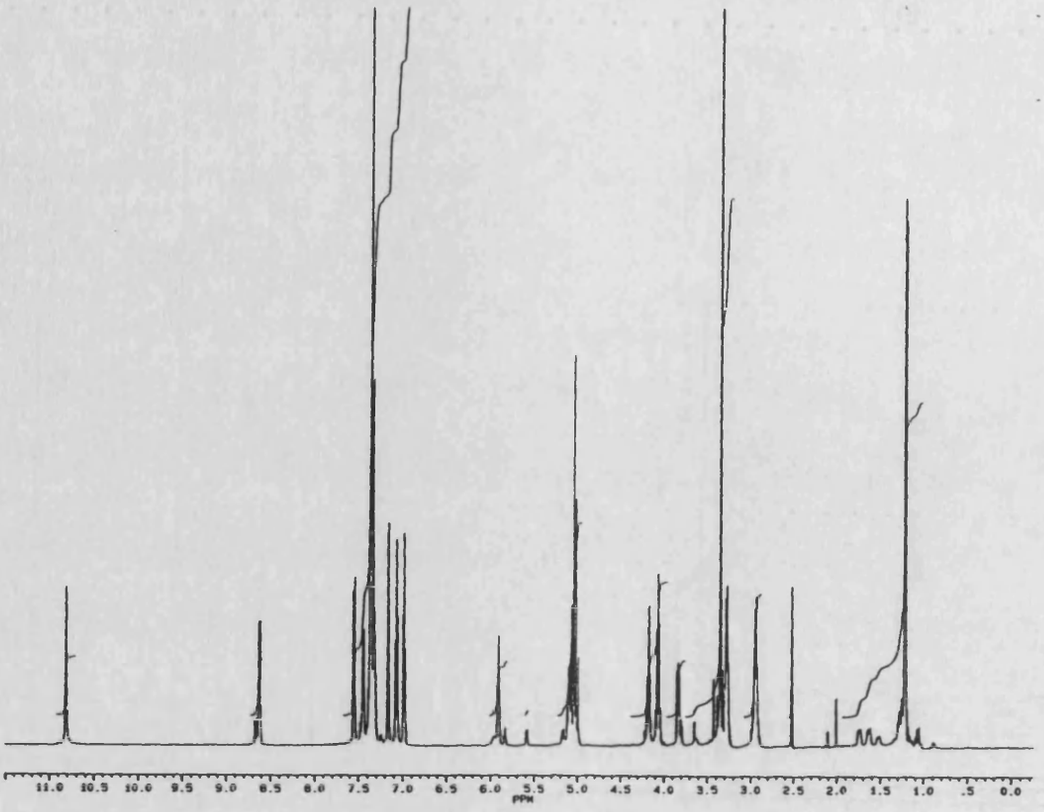


Fig. 7

Tripeptide ; [3′S]-N-[2-[[3-[[2-(3-indolyl)ethyl]amino]-4,5-dihydro-4-isoxazolylamino]-1-methyl-2-oxoethyl]-carbamic acid, phenylmethyl ester (57)



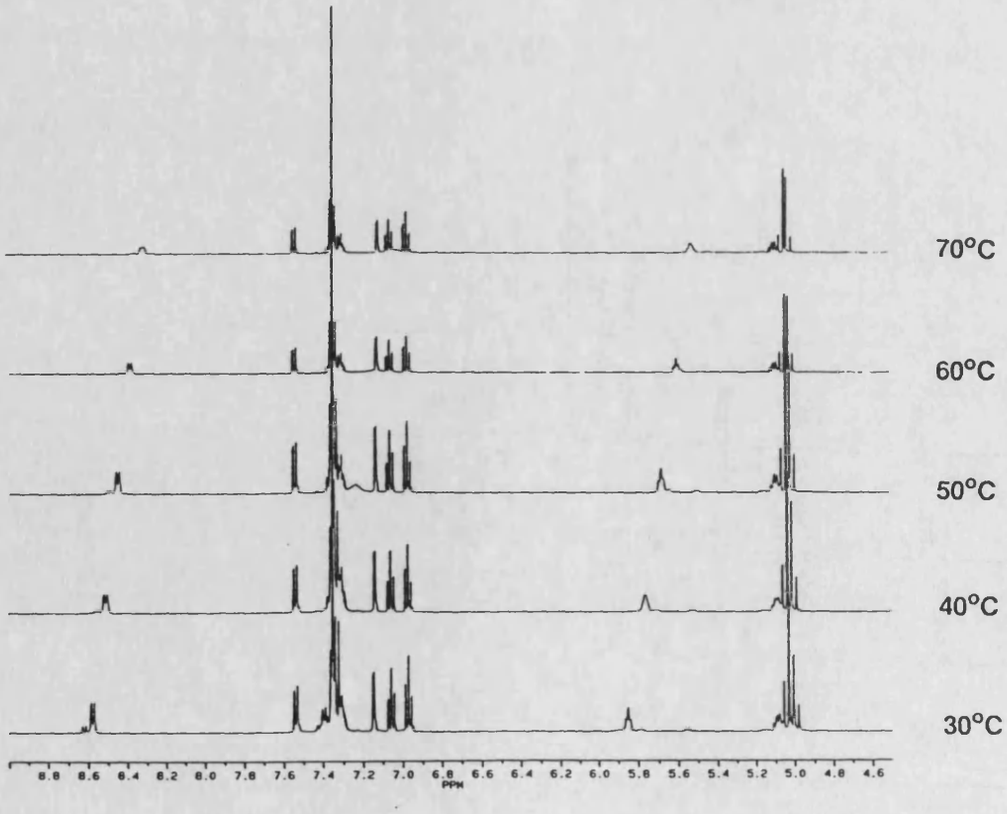
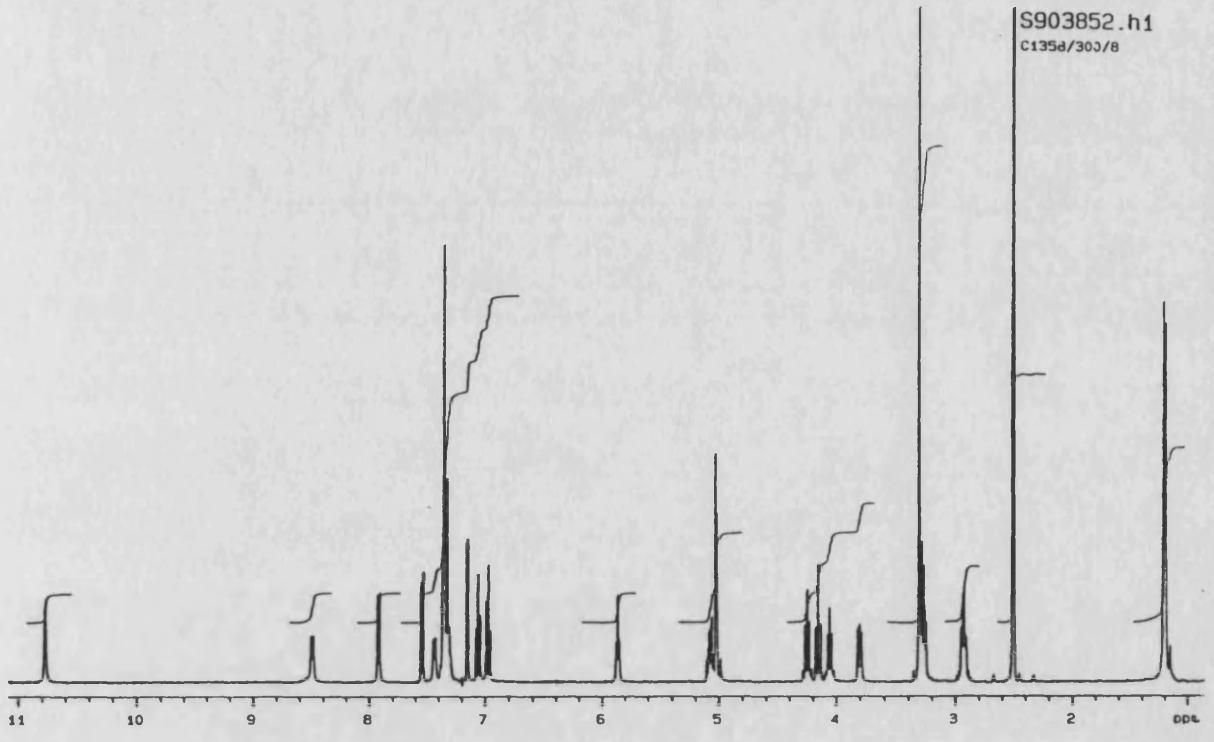
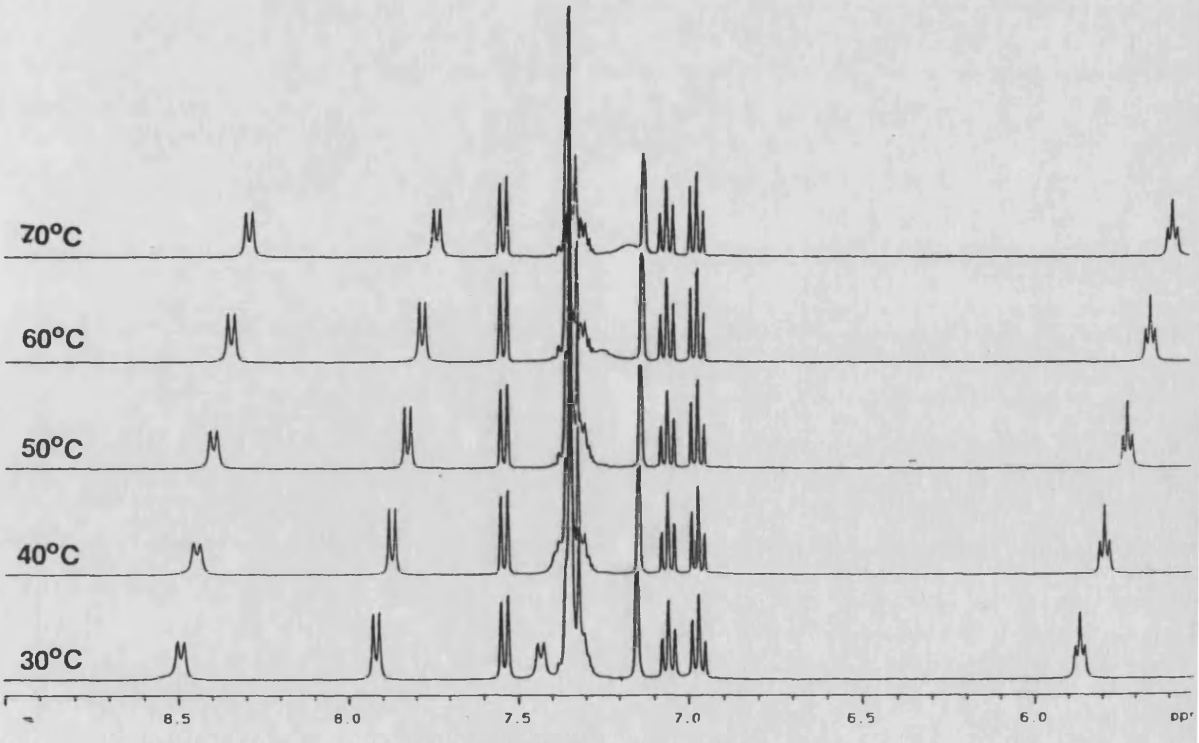


Fig. 8

Tetrapeptide : [3′S]-N-[2-[2-[[3-[[2-(3-indolyl)ethyl]amino]-4,5-dihydro-4-isoxazolylamino]-1-methyl-2-oxoethyl]amino]-1-methyl-2-oxoethyl]carbamic acid, phenylmethyl ester (59)





1.5 Summary

In Chapter 1 we have described the synthesis and reactivity of imidoyl halides and mesylates derived from (R)-cycloserine. Our goal was to generate a novel constrained amino acid mimetic (corresponding to L-glycine) for incorporation into peptides to "induce" a γ-turn conformation. While we have had some success this chemistry and its subsequent application has been limited by a number of factors:-

1. The bulkiness of the protecting groups at C-4 sterically hinders nucleophilic attack at the adjacent C-3 position.

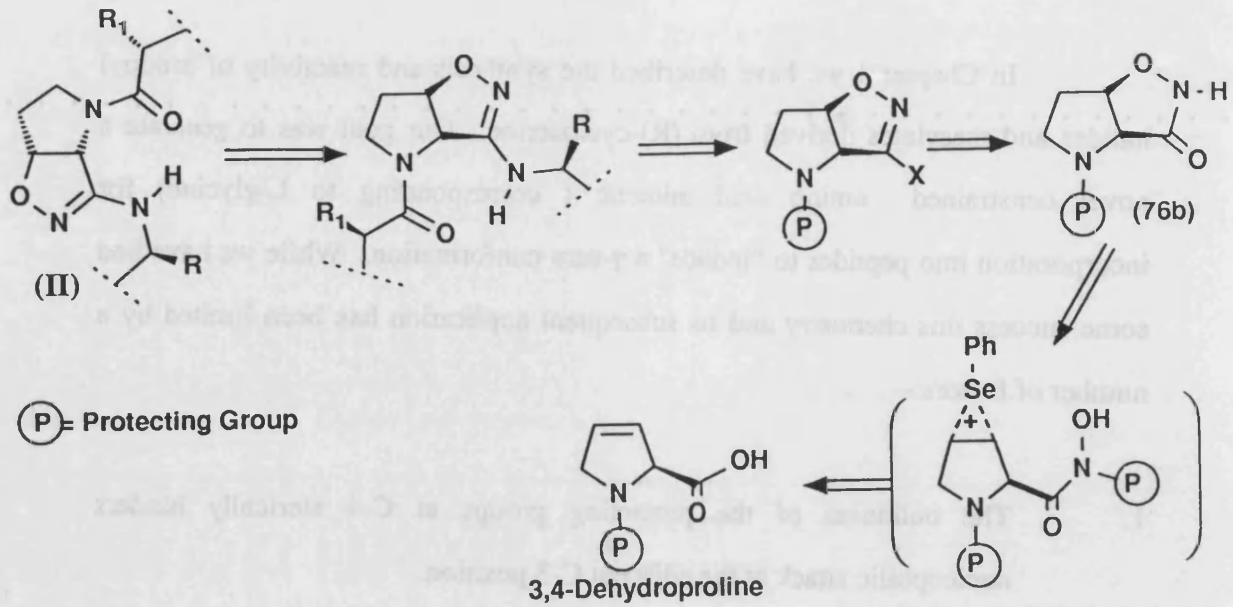
2. The electrophilic nature of the protecting groups compared with that of the imidoyl halides leads to reaction at this alternative site.

3. The stability of imidoyl halides towards amino esters in displacement reactions limited the range of substrates that we were able to make available.

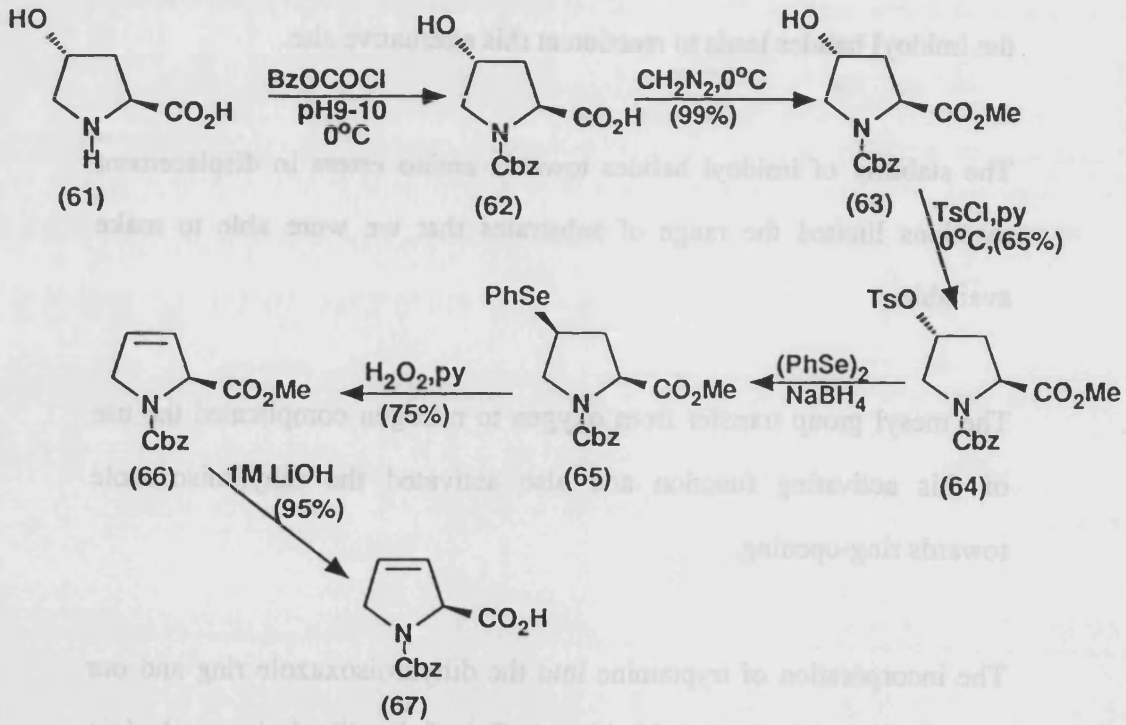
4. The mesyl group transfer from oxygen to nitrogen complicated the use of this activating function and also activated the dihydroisoxazole towards ring-opening.

The incorporation of tryptamine into the dihydroisoxazole ring and our ability to extend a "peptide-like" chain through C-4 of the dihydroisoxazole (see Scheme 28a) has endorsed the possible use of the dihydroisoxazole ring as a heterocyclic constrained glycine unit.

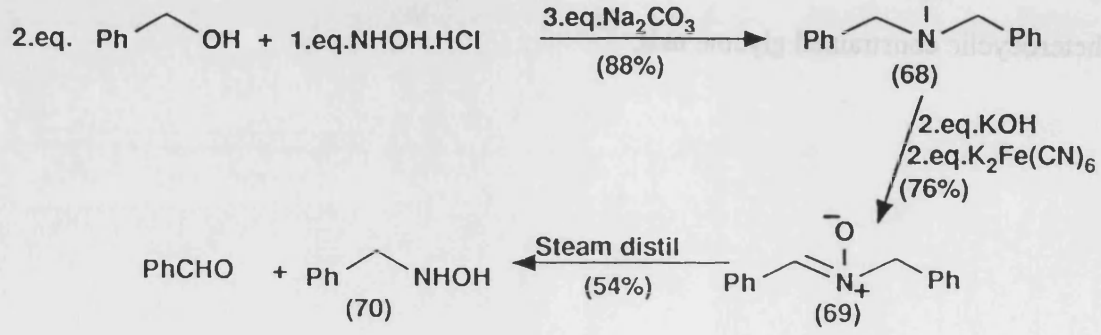
Scheme 29



Scheme 30



Scheme 31



CHAPTER 2

2. *Synthesis of (cis)-Hexahydro-2H-pyrrolo[2,3-d]isoxazolidine-3-one*

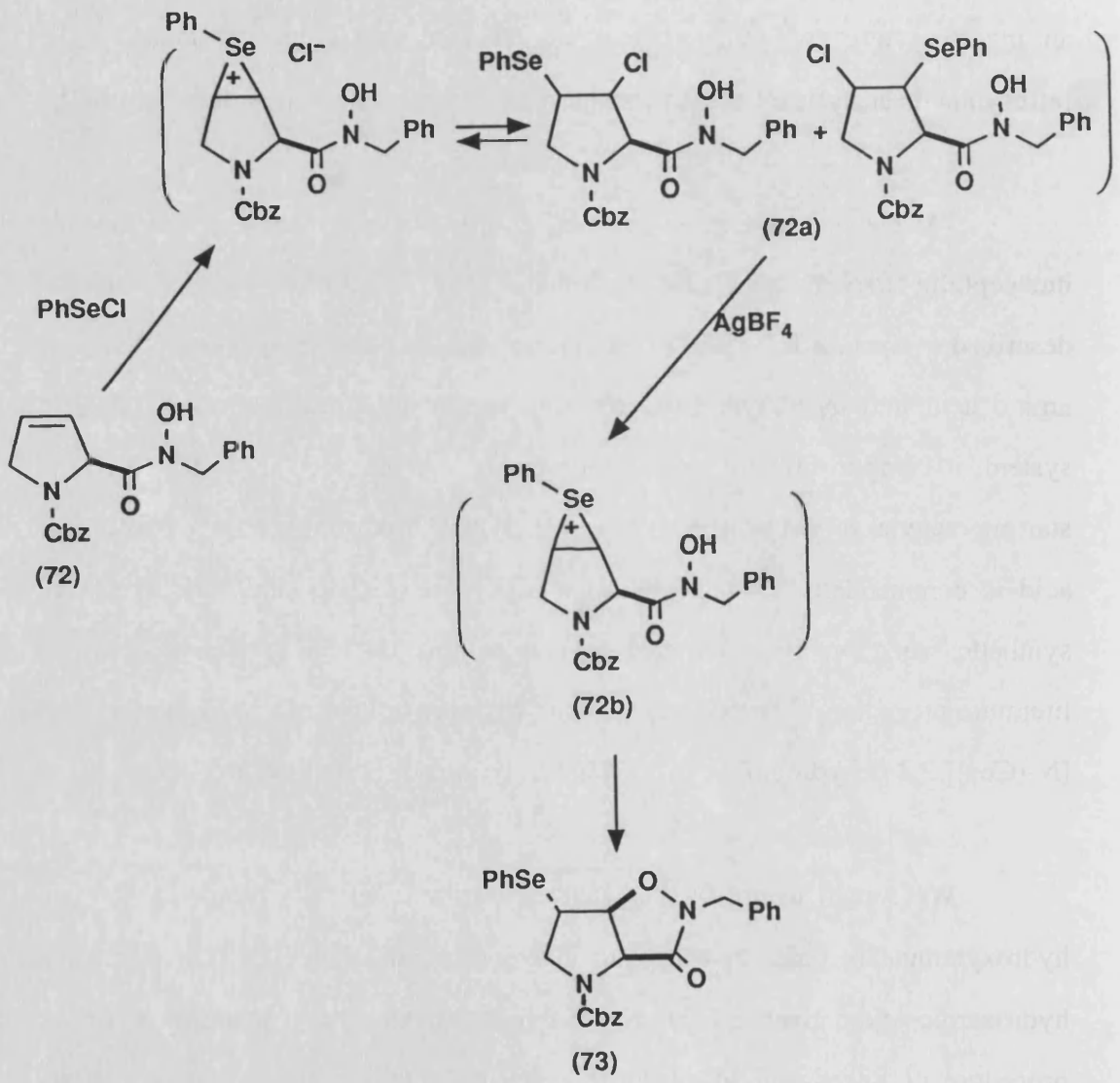
As outlined in the Introduction (*Section 2.0*), bicycle (**II**) has torsion angles ϕ = -65.9° and ψ = 62.2°. This is in agreement with an inverse γ-turn (ϕ = -70° to -80° and ψ = +60° to +70°) and this structure would be expected to induce an inverse γ-turn conformation once incorporated into a peptide chain. The retrosynthetic analysis for the (S)-enantiomer of bicycle **II** is shown in Scheme 29.

We planned to activate pyrrolo[2,3-d]isoxazolidine-3-one (**76b**) towards nucleophilic attack by generating the imidoyl halide function as has been already described in *Section 1.1.* We hoped that our lack of success in incorporating an amino acid into the dihydroisoxazole ring would not prevail upon the bicycle system, it being a different structure altogether. As outlined in Scheme 29, the starting material for the synthesis of bicycle (**II**) is 3,4-dehydroproline. This amino acid is commercially available but at a high cost (£90g^{-1}) and for large scale synthetic work, we were required to prepare this material ourselves, using a literature procedure[106] based on (2S,4R)-4-hydroxyproline (**61**). The synthesis of [N-(Cbz)]-3,4-dehydroproline (**67**) using this procedure is outlined in Scheme 30.

We sought to use N-benzylhydroxylamine (**70**) as a protected form of hydroxylamine in order to couple to dehydroproline to give the corresponding hydroxamic acid derivative (**72**). N-Benzylhydroxylamine was prepared using the procedure of Sneed and Jones[107](Scheme 31) and this proved to be a more reliable method in our hands than the alternative route based on the reduction of benzyloxime with diborane in THF.[108]

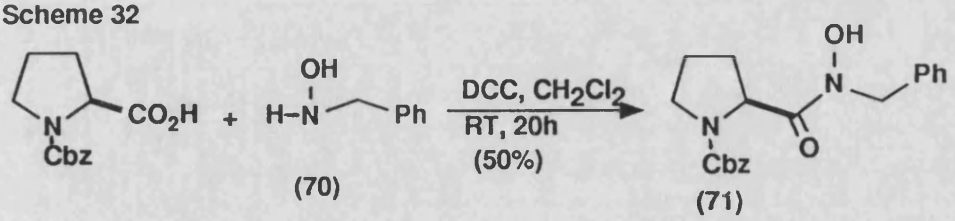
The coupling of benzylhydroxylamine (**70**) with [N-(Cbz)]-(L)-proline

Scheme 32b



was examined as a model for the route based on 3,4-dehydroproline. The desired hydroxamic acid (**71**) was obtained in 50% yield as shown in Scheme 32.

Scheme 32

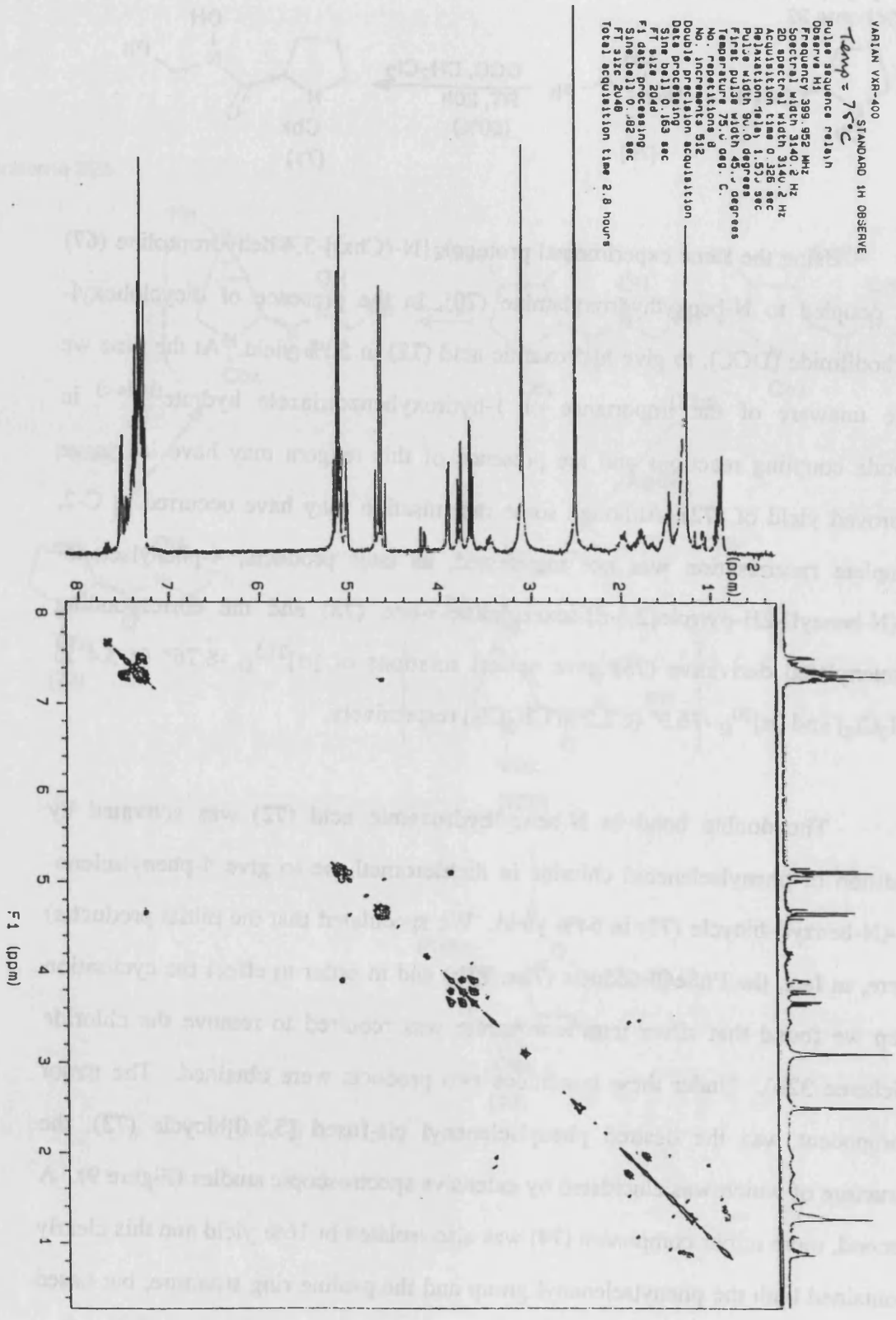


Using the same experimental protocol, [N-(Cbz)]-3,4-dehydroproline (**67**) was coupled to N-benzylhydroxylamine (**70**), in the presence of dicyclohexyl--carbodiimide (DCC), to give hydroxamic acid (**72**) in 50% yield. At the time we were unaware of the importance of 1-hydroxybenzotriazole hydrate[109a-c] in peptide coupling reactions and the presence of this reagent may have led to an improved yield of (**72**). Although some racemisation may have occurred at C-2, complete racemisation was not manifested, as later products, 4-phenylseleno--2-(N-benzyl)-2H-pyrrolo[2,3-d]isoxazolidine-3-one (**73**) and the corresponding deselenylated derivative (**75**) gave optical rotations of $[\alpha]^{21.5}_{D}$ -8.76° (c 3.4 in CH_2Cl_2) and $[\alpha]^{20}_{D}$ -76.9° (c 2.2 in CH_2Cl_2) respectively.

The double bond in N-benzylhydroxamic acid (**72**) was activated by addition of phenylselenenyl chloride in dichloromethane to give 4-phenylseleno--2-(N-benzyl)-bicycle (**73**) in 64% yield. We speculated that the initial product(s) were, in fact, the PhSeCl adducts (**72a**, **72b**) and in order to effect the cyclisation step we found that silver tetrafluoroborate was required to remove the chloride (Scheme 32b). Under these conditions two products were obtained. The major component was the desired phenylselenenyl cis-fused [3.3.0]bicycle (**73**), the structure of which was elucidated by extensive spectroscopic studies (Figure 9). A second, more minor component (**74**) was also isolated in 16% yield and this clearly contained both the phenylselenenyl group and the proline ring structure, but based on the ^1H NMR spectra, the benzyloxycarbonyl group at δ 5.00 + 5.12 (obtained

Fig. 9

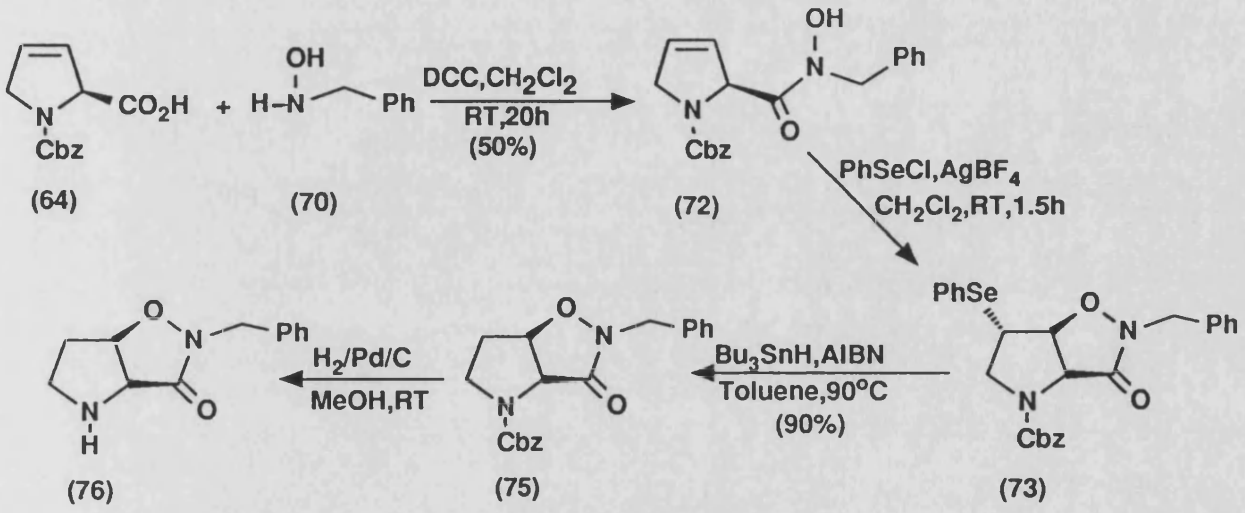
4-(Phenylseleno)-(cis)-Hexahydro-2H-pyrrolo[2,3-d]isoxazoline-3-one (73)



from compound (**72**)) appeared to have been lost. The AB quartet at lower field (δ 4.60 + 4.72) suggested the presence a simple $OC\underline{H_2}Ph$ residue and a molecular ion of 465 (M^+ + H) (44 mass units less than the corresponding 4-phenylselenenyl bicycle (**73**) (509 (M^+ + H)) was observed. Based on this evidence it is clear that the minor component is <u>not</u> the isomeric [3.2.1] bridged-bicycle (**79**) and the structure of this other product has not been identified.

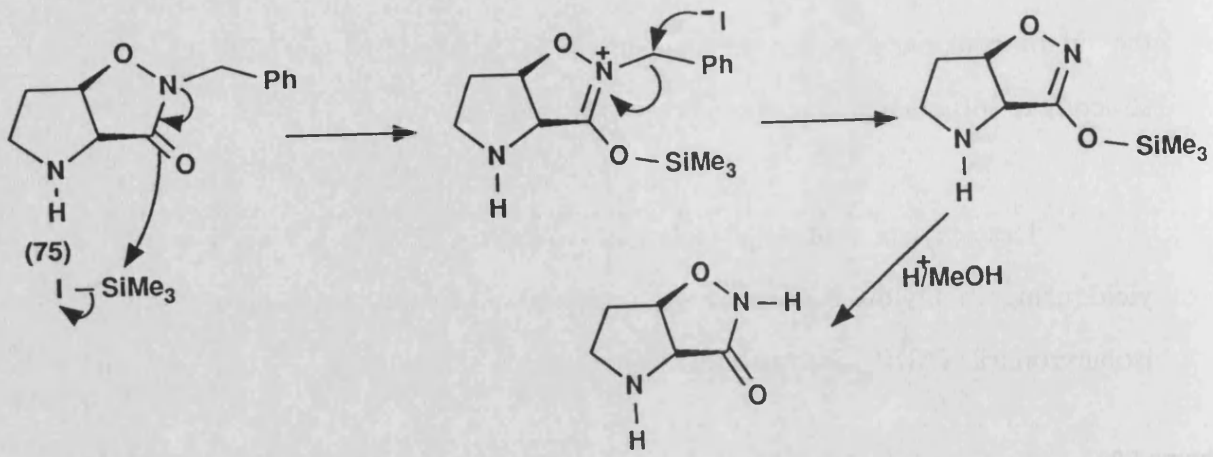
Deselenylation of 4-(phenylseleno) bicycle (**73**) was achieved in 91% yield using tributyltin hydride in the presence of catalytic amount of azobis-isobutyronitrile (AIBN) in toluene at reflux (Scheme 33a).

Scheme 33a

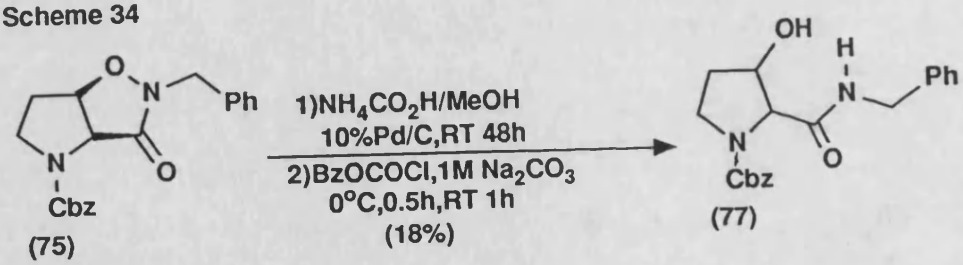


Complete deprotection of bicycle (**75**) using atmospheric hydrogenation in the presence of either 10% palladium over charcoal catalyst or 20% palladium hydroxide, could not be achieved. In the event, only the N-benzyloxycarbonyl group was removed to afford 2-[N-benzyl]pyrrolo[2,3-d]isoxazolidine-3-one (**76**) in 73 and 61% yields respectively. Other attempts to cleave both protecting groups simultaneously were also unsuccessful and in all cases only the N-benzyloxycarbonyl group was removed to give bicycle (**76**). These efforts included the use of trifluoromethanesulphonic acid and anisole,[(104)] phase transfer hydrogenation with ammonium formate and 10% palladium over charcoal

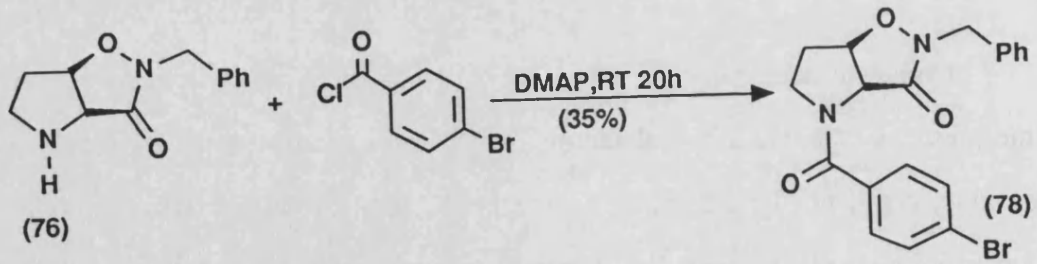
Scheme 33b



Scheme 34



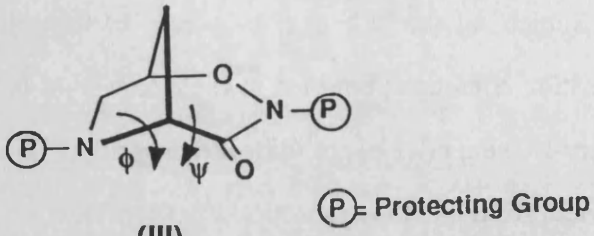
Scheme 35



catalyst,[110] and the use of iodotrimethylsilane.[111] Iodotrimethysilane is known to cleave esters, ethers and carbamates,[111] so the removal of the N-benzyloxycarbonyl group was of no surprise. We had envisaged that the oxygen atom in the carbonyl function in bicycle (**75**) would be silylated and that iodide ion would attack the N-benzyl group as illustrated in Scheme 33b.

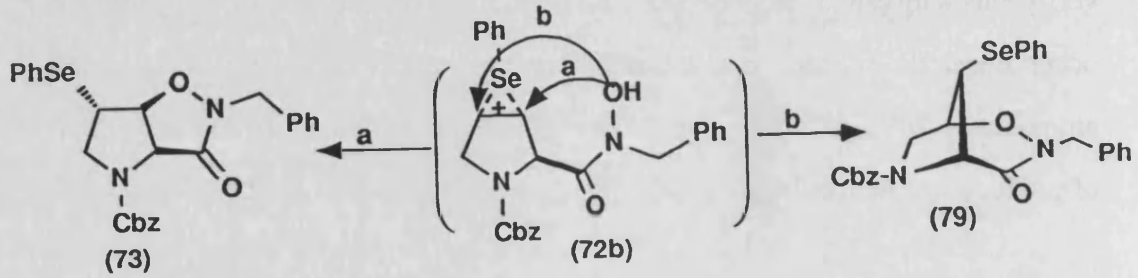
Subjecting the N-benzyl bicycle (**76**) for long periods to phase transfer hydrogenation conditions only led to the cleavage of N-O bond in the bicyclic ring. 3-Hydroxy-N-(benzyl)-2-pyrrolidinecarboxamide (**77**) was subsequently isolated in 18% yield from the above reaction after reprotection of the proline nitrogen with benzyl chloroformate. The overall reaction sequence is outlined in Scheme 34 and although a *cis* relationship between C-2 and C-3 would be expected, we did not verify this and cannot exclude the possibility that epimerisation of C-2 did not occur under the reaction conditions. Treating bicycle (**76**) with sodium-liquid ammonia at -78°C in THF[112] was also unsuccessful leading to a complex mixture of products and with only a low (10%) recovery of starting material.

It is evident that the N-benzyl protecting group cannot be removed under normal mild hydrogenation conditions without the cleavage of the weak N-O bond in bicycle (**76**). Bicycle (**76**) was nevertheless N-acylated with 4-bromobenzoyl chloride in the presence of 4-dimethylaminopyridine in THF (Scheme 35), in the hope of obtaining crystals for X-ray crystallographic analysis and to aid full characterisation. Unambiguous assignment of structure and stereochemistry could then be made as all prior intermediates had been oils. To date, we have obtained a satisfactory elemental analysis for the acylated bicycle (**78**) but, as yet, crystals for X-ray analysis have not been produced. Further synthetic work related to 2H-pyrrolo[2,3-d]isoxazolidine-3-one (**76**) and its incorporation into a peptide chain has been postponed.



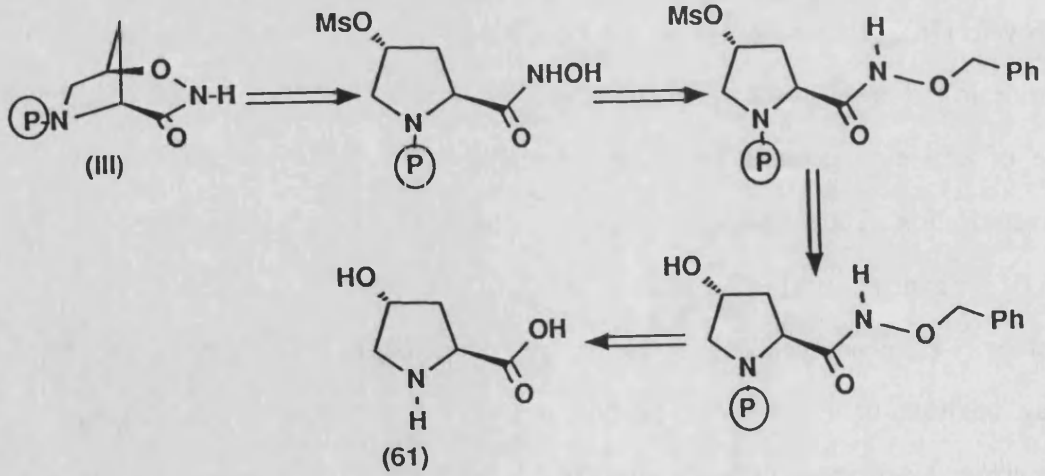
(III)

Scheme 36



(73) (72b) (79)

Scheme 37



(III) (61)

CHAPTER 3

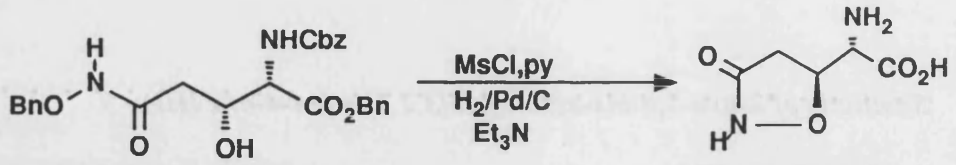
3. *Synthesis of 2-oxa-3,6-bis-azabicyclo[3.2.1]octan-4-one (III)*

The [3.2.1]bicycle (**III**) is another heterocyclic constraint unit that we aimed to synthesise. The torsion angles of this unit have been calculated by CONDOR to be ϕ = -95.8° and ψ = +113.9°. This molecular framework, based on bicycle (**84**), would therefore exert a very different type of constraint from that of the bicycle (**II**) described above. We had hoped that, during the electrophile--mediated cyclisation of the 3,4-dehydroproline derivative (**72**) with phenyl--selenenyl chloride and silver tetrafluoroborate, some of the bridged bicycle (**79**) may have formed i.e. *path b* in Scheme 36. In the event the only isomer obtained corresponded to (**73**) (*path a*) so we sought to synthesise the bridged bicycle (**84**) by an alternative route from (2S,4R)-4-hydroxylproline. The retrosynthetic analysis of bicycle (**84**) is shown in Scheme 37.

3.11. *Synthesis and Attempted Cyclisation of (4R)-Methanesulphonyloxy--N-(hydroxy)-(2S)-pyrrolidinecarboxamide*

Using Schotten-Baumann conditions, the amine function of (2S,4R)--4-hydroxyproline (**61**) was protected with benzylchloroformate. O-Benzyl-hydroxylamine (a latent hydroxylamine unit) was then incorporated by reacting the N-protected hydroxyproline (**62**) with O-benzylhydroxylamine in the presence of DCC to give (**80**) in 52% yield. The C-4 hydroxyl function was then mesylated under standard conditions and mesylate (**81**) was deprotected by atmospheric hydrogenolysis in the presence of 10% palladium over charcoal as catalyst, to give (4R)-methanesulphonyloxy-N-(hydroxy)-(2S)-pyrrolidinecarboxamide (**82**) in 86% yield.

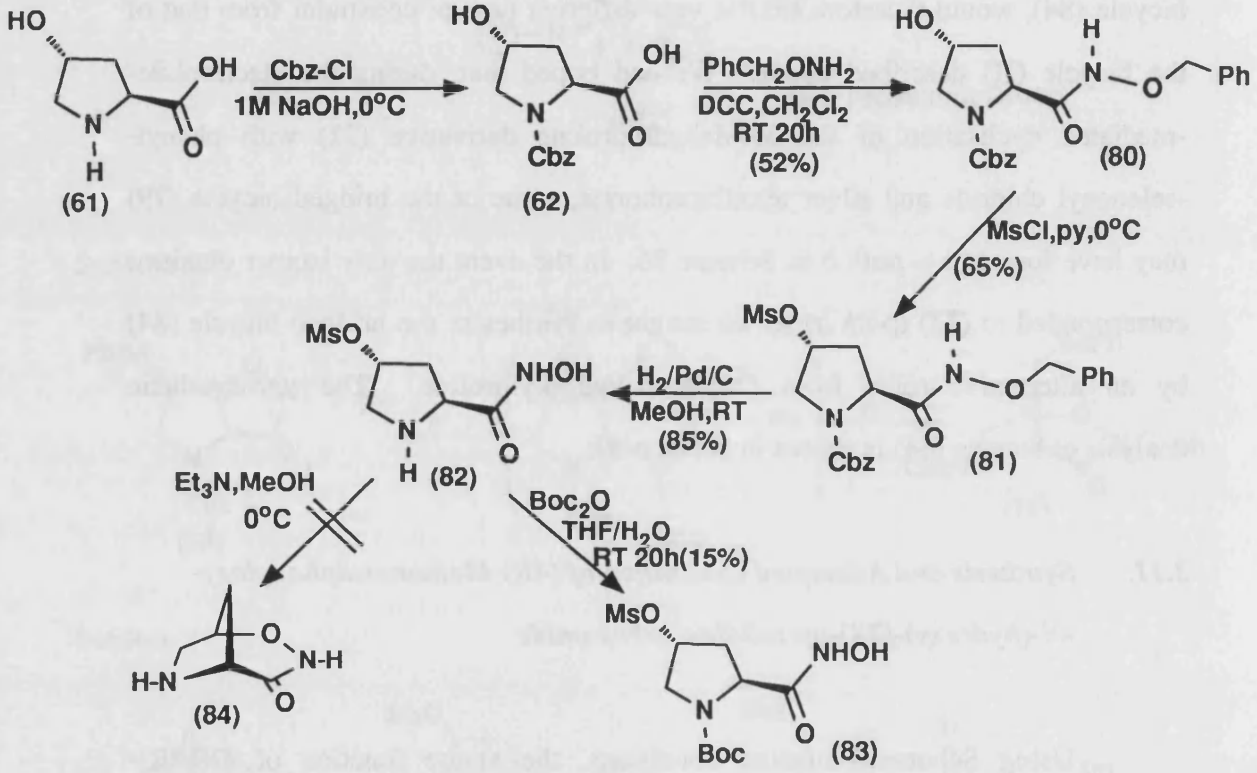
Scheme 38



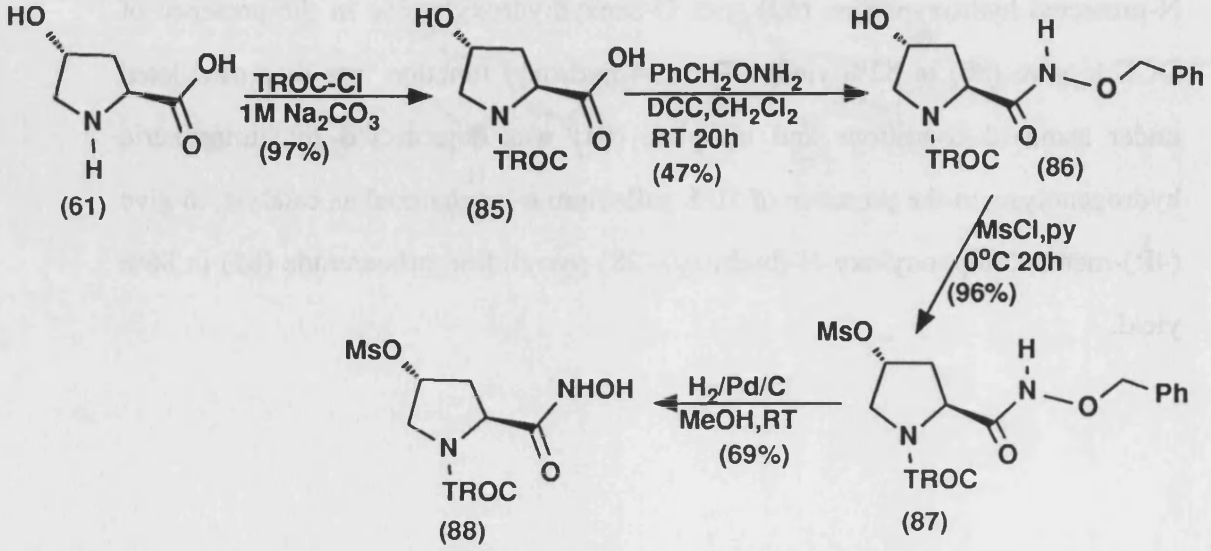
2-amino-3-hydroxyglutaric acid benzyl ester(δ- N-benzyloxyamide)

Tricholomic acid

Scheme 39



Scheme 40

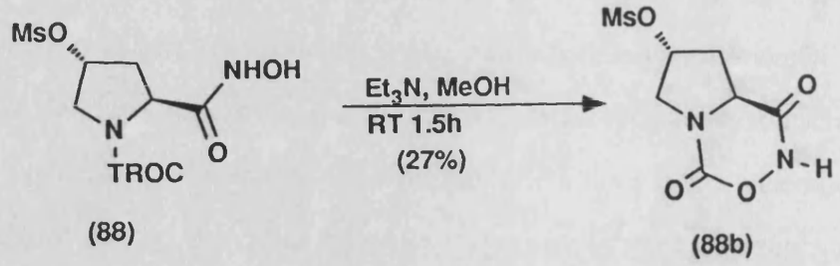


Attempts to cyclise hydroxamic acid (82) using methods very similar to those reported by Hanessian[115] for the synthesis of tricholomic acid from d,l-*threo*-2-amino-3-hydroxyglutamic acid benzyl ester (δ-N-benzyloxyamide) (Scheme 38) i.e. stirring at 0°C in the presence of triethylamine in water, were unsuccessful. Other efforts directed towards this end, such as changing the solvent and varying reaction conditions were also unfruitful. Hydroxamic acid (82) was a very polar compound and it was difficult to follow any reaction by TLC. Attempts to reprotect the nitrogen atom of the proline ring with either benzyl chloroformate or with di-*tert*-butyldicarbonate (BOC_2O) were equally unsuccessful, although some reprotected product (83) in only 15% yield was isolated with BOC_2O. It is possible that hydroxamic acid (82) is polymerising in the reaction conditions applied to either achieve cyclisation or affect reprotection (Scheme 39).

3.12. Synthesis and Cyclisation Reactions of 1-[N-(2,2,2-Trichloroethyloxy-carbonyl)]-(4R)-Methanesulphonyloxy-N-(hydroxy)-(2S)-pyrrolidine-carboxamide

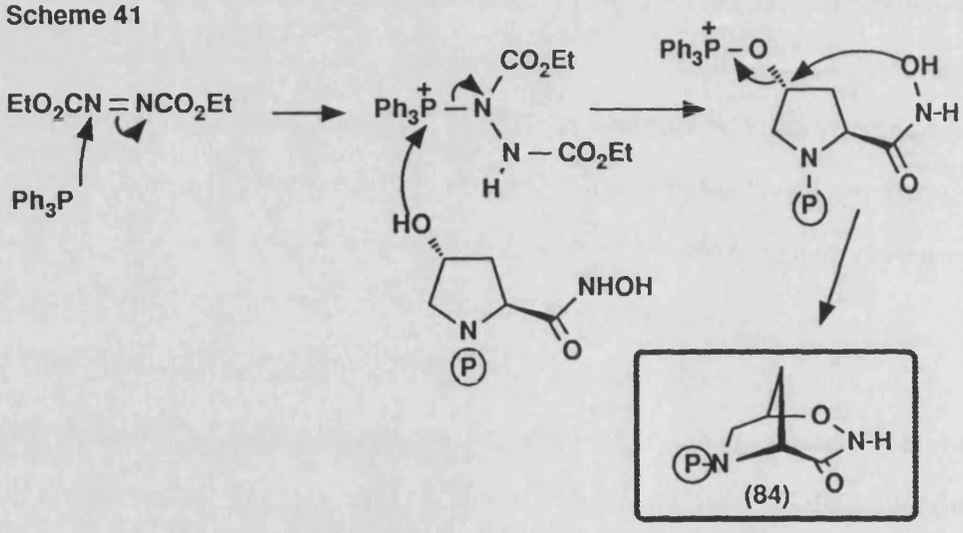
Given the above observations we sought an alternative protecting group that would be stable under hydrogenolysis conditions. 2,2,2,-Trichloroethyl--oxycarbonyl (TROC) was chosen since this residue is easily removed in the presence of zinc in acetic acid.[89a] (2S,4R)-4-Hydroxyproline was N-protected with 2,2,2-trichloroethyl chloroformate in the presence of 1M aqueous sodium bicarbonate solution to give (85) in 97% yield which was subsequently converted to [N-(TROC)]-(4R)-methanesulphonyloxy-N-(hydroxy)-(2S)-pyrrolidinecarbox-amide (88) using the same experimental conditions established earlier for the synthesis of the [N-(Cbz)] derivative (82). The overall yield of (88) was 77% and the sequence is illustrated in Scheme 40.

Scheme 40b

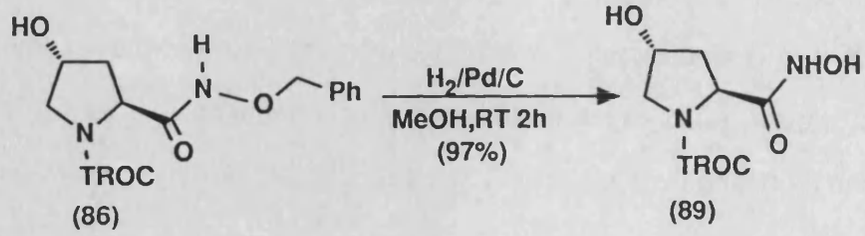


(88) (88b)

Scheme 41



Scheme 42



(86) (89)

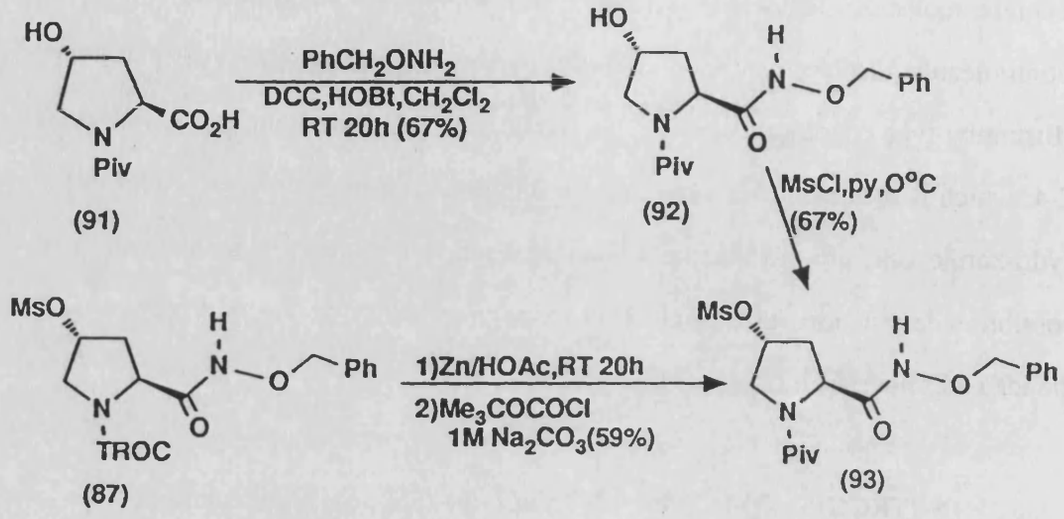
Mesylate (88) was stirred with triethylamine in methanol in an attempt to achieve cyclisation to generate the [3.2.1] bridged bicycle corresponding to that required for (III), but instead displacement of the trichloroethyloxy group occurred, to give oxadiazine derivative (88b) (Scheme 40b). Oxadiazine (88b) was characterised only by spectroscopic methods; ^{1}H, ^{13}C NMR and IR only, an accurate molecular ion was not found. We envisaged an alternative mode of intramolecular displacement involving (89) by activating the hydroxyl at C-4 under Mitsunobu-type conditions.[116a-c] This would give the alkoxyphosphonium salt at C-4, which is then susceptible to nucleophilic attack, in this case by the OH in the hydroxamic acid group (Scheme 41). Although, we could foresee a number of possible side reactions that would lead to polymerisation of the pyrrolidine ring, the idea was nevertheless pursued.

[N-(TROC)]-(4R)-Hydroxyl-N-(hydroxy)-(2S)-pyrrolidinecarboxamide (89) was prepared by debenzylation of (4R)-hydroxyl-N-(phenylmethoxy)-(2S)--pyrrolidinecarboxamide (86) (Scheme 42). However, treating (89) with triphenylphosphine and diethylazodicarboxylate, only a complex mixture was obtained and this route was abandoned.

3.13. Synthesis of N-(Pivaloyl)-3-(Hydroxyimino)-2-oxa-5-azabicyclo[2.2.1]-heptane (95)

Evidently, the carbamate protecting group was interfering with the cyclisation of 1-[N-(TROC)]-(4R)-methanesulphonyloxy-N-(hydroxy)-(2S)--pyrrolidinecarboxamide (88) as described in *Section 3.12*. Pivaloyl chloride was then chosen as the N-protecting residue for (2S,4R)-4-hydroxyproline. [N-(Pivaloyl)]-(2S,4R)-4-hydroxyproline (91) was prepared using similar procedure described for FMOC N-protection of amino acids.[86] N-pivaloyl (91) was then converted to the corresponding mesylate (93) using the same reaction

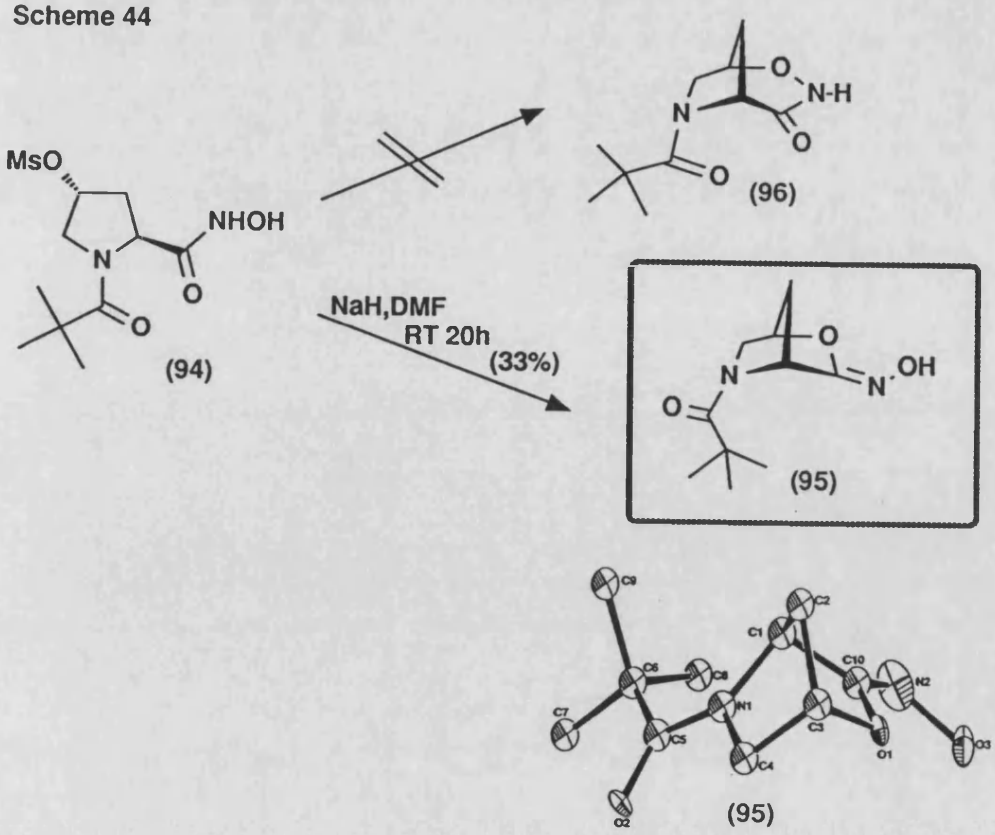
Scheme 43


HO
CO2H
N
Piv
(91)
PhCH2ONH2
DCC,HOBt,CH2Cl2
RT 20h (67%)
HO
H
N
O
Ph
N
Piv
O
(92)
MsCl,py,O°C
(67%)
MsO
H
N
O
Ph
N
TROC
O
(87)
1)Zn/HOAc,RT 20h
2)Me3COCOCl
1M Na2CO3(59%)
MsO
H
N
O
Ph
N
Piv
O
(93)

conditions employed for the synthesis of the N-Cbz derivative (**81**), in 63% overall yield (Scheme 43). Similarly, [N-(TROC)] carboxamide (**87**) could be converted to (**93**) by deprotection of the TROC group (Zn/HOAc) and then reprotection (pivaloyl chloride in pyridine) in 59% overall yield.

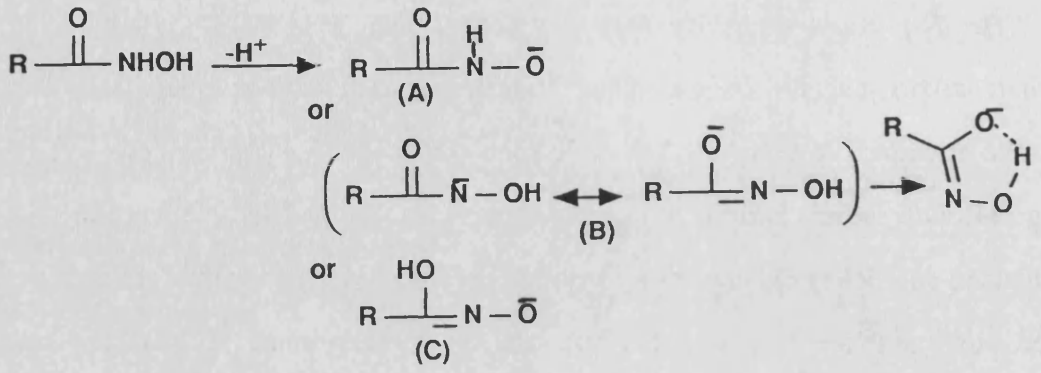
The N-pivaloyl derivative (**93**) was debenzylated (H_2, 10% palladium over charcoal) to give the corresponding hydroxamic acid (**94**) in quantitative yield. We attempted to carry out the cyclisation step under basic conditions by treating (**94**) with sodium hydride in dimethylformamide. A cyclised product (**95**) was obtained in 33% yield but X-ray crystallographic analysis of this product revealed its structure to be that of the 3-(hydroxyimino)-2-oxa-5-aza-bicyclo[2.2.1]heptane (**95**) and not the expected [3.2.1]bridged bicycle (**96**) (Scheme 44).

Scheme 44

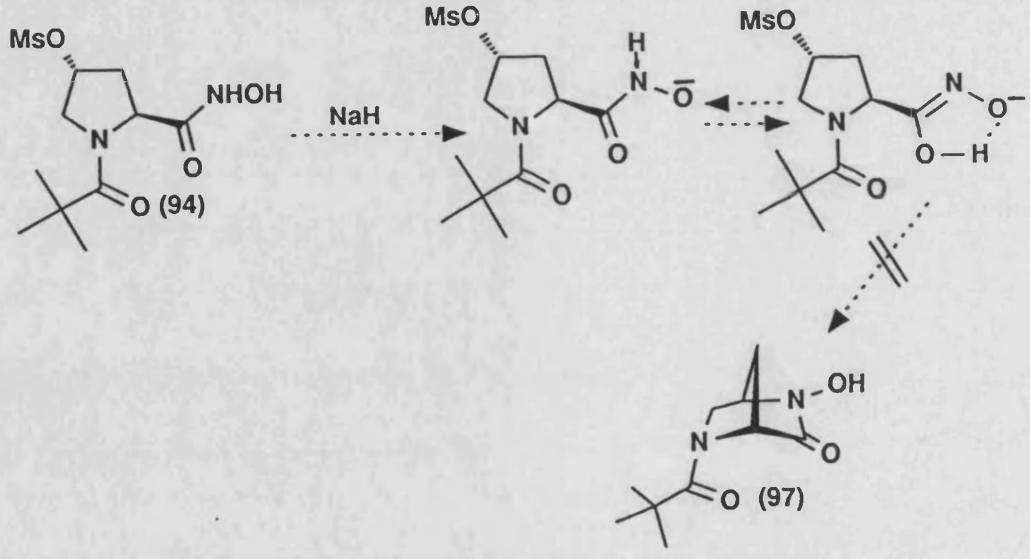


(95)

Scheme 45



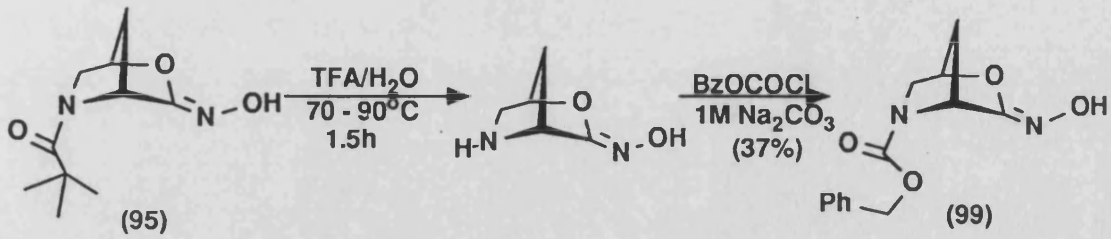
Scheme 46



It is apparent that cyclisation has occurred via the carbonyl oxygen in a 5,5 (enol-exo) exo-tet fashion rather than in a 5,6 exo-tet from the oxygen in the hydroxamic acid (NOH) group,[117a,b] but both pathways are favoured according to Baldwin's Rules of Ring Closure.[114a,b] It has been reported that five-membered ring formation is generally much faster than the corresponding closure to give the six-membered ring.[113] Alkylations of hydroxmates generally occur via the oxygen of NOH[118] and the anion of hydroxamate is known to prefer to exist in tautomer form (**B**) rather than (**A**) or (**C**)[120] with tautomer (**B**) stabilised by an intramolecular hydrogen bond[121] (Scheme 45). Hence, for a 6 exo-tet cyclisation to occur in (**94**), this hydrogen bond would have to be broken. Similarly, one can envisage cyclisation via the nitrogen in the hydroxamic acid (**94**) to give the [2.2.1] bicycle (**97**), however, this process would require a 5,5 (enol-endo) exo-tet cyclisation mode which is a disfavoured pathway[117] (Scheme 46).

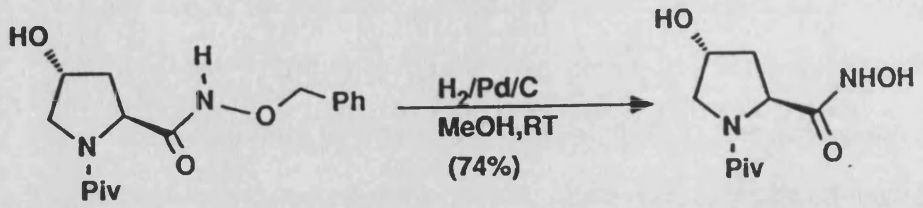
The N-pivaloyl protecting group has been reported to be effectively removed under acid hydrolysis condition (reflux in TFA).[122] Subsequent treatment of N-(pivaloyl) bicyclo[2.2.1]heptane (**95**) with aqueous TFA ((4:1) at reflux), followed by reprotection with benzyl chloroformate afforded the corresponding N-(benzyloxycarbonyl)-azabicycle (**99**) in 37% yield (Scheme 47).

Scheme 47



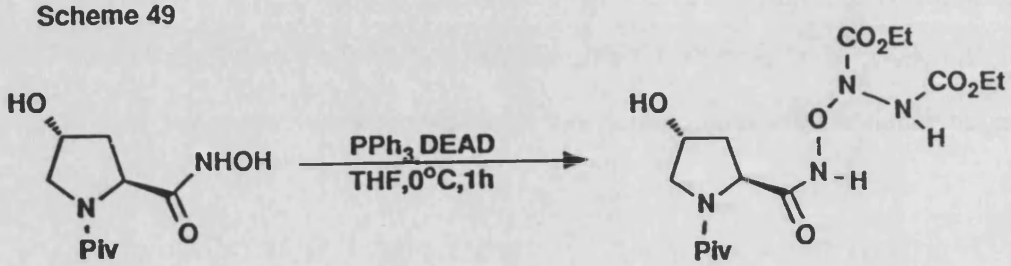
An alternative route to the [3.2.1]bridged bicycle (**96**) would be to activate the hydroxyl function at C-4 of the N-(pivaloyl) hydroxamic acid (**100**) by treatment of triphenylphosphine and diethylazodicarboxylate, as described in *Section 3.12*. [N-(pivaloyl)]-(4R)-hydroxyl-N-(hydroxy)-(2S)-pyrrolidinecarbox-

Scheme 48



(92) (100)

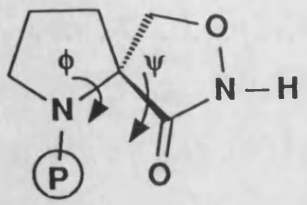
Scheme 49



(100) (101)

amide (**100**) was prepared by debenzylation of N-(phenylmethoxy)-(2)-pyrrolidine-carboxamide (**92**) (H_2,Pd/C) (Scheme 48). Treatment of (**100**) with triphenylphosphine and DEAD (THF, 0°C, 1h) gave an adduct of (**100**) that had incorporated DEAD. We propose that the hydroxamic acid (NOH) function in (**100**) had reacted with the DEAD reagent before triphenylphosphine, to give the adduct (**101**) and this assignment is consistent with spectroscopic data obtained (^1H, ^{13}C NMR and IR only, no molecular ion was observed) (Scheme 49). This result was not a complete surprise since hydroxamic acid has a pKa of 9[120] and repeating this reaction by first generating the PPh_3/DEAD complex followed by addition of hydroxamic acid (**100**) still afforded the same adduct (**101**) in 46% yield.

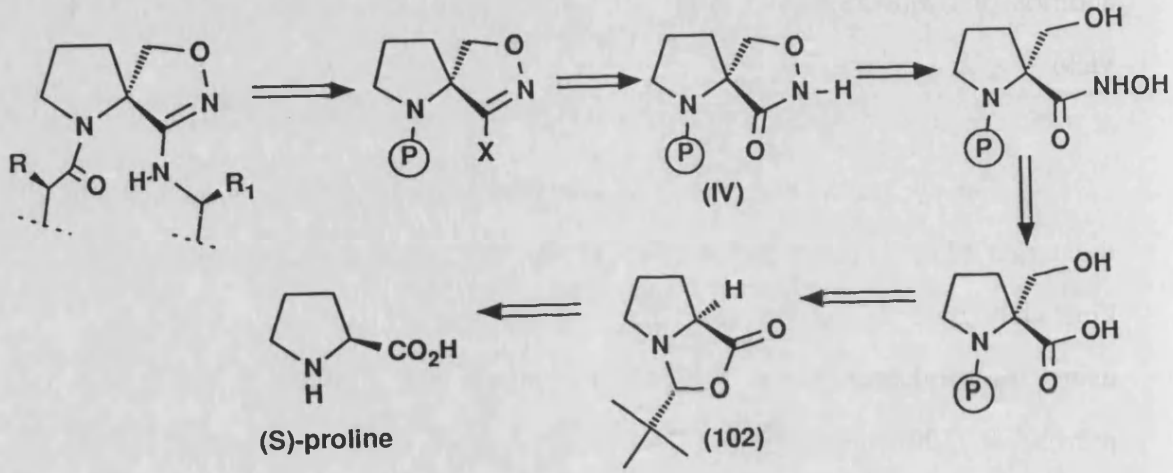
The cyclisation of hydroxamic acid (**100**) does not offer a viable route to the target [3.2.1]bridged bicycle (**96**) as the "5,5" ring formation is favoured kinetically over that of the "5,6" ring. Alternative intramolecular cyclisations using triphenylphosphine and DEAD to activate the 4-hydroxyl function in pyrrolidine (**100**) was also unfruitful and further effort on the synthesis of bicycle (**III**) was thus abandoned.



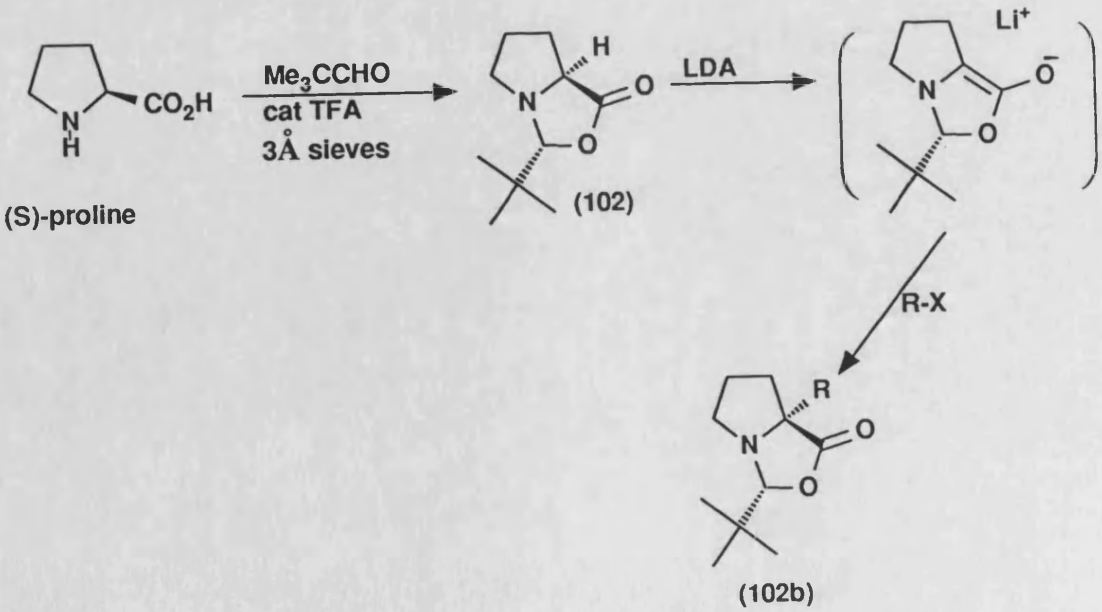
P = Protecting Group

(IV)

Scheme 50



Scheme 51



CHAPTER 4

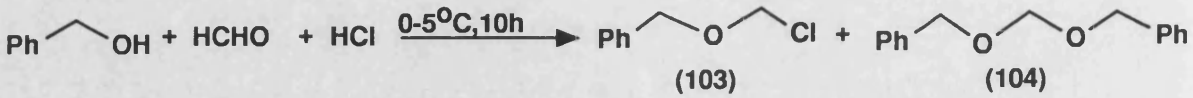
4. *Synthesis of 4,4-(2-pyrrolidine)-4-isoxazolidine-3-one (IV)*

The final peptide mimetic that we have examined is the spiro derivative (**IV**) which has been calculated by CHEMX to have torsion angles (ϕ=-60° and ψ=-60°) corresponding to that of a right-handed α-helix. The retrosynthetic analysis of this system is shown in Scheme 50, where (S)-proline is the starting material. We needed to be able to alkylate proline stereospecifically at C-2 with a "$^{\oplus}CH_2OH$" equivalent. Seebach has developed a method for the asymmetric α-alkylation of (S)-proline[123]. This is based on the condensation of pivaldehyde with (S)-proline to give a single diastereoisomer of 2-*tert*-butyl-1-aza-3--oxabicyclo[3.3.0]octan-4-one (**102**). This intermediate can be deprotonated with lithium diispropylamine (LDA) to give a chiral, non-racemic enolate which can react with electrophiles with retention at the C-2 centre of the pyrrolidine ring (Scheme 51).

(S)-Proline was condensed with a 2.5 fold excess of pivaldehyde in the presence of catalytic amount of trifluoroacetic acid, with removal of water (3Å molecular sieves). This gave bicyclo[3.3.0]octan-4-one (**102**) in quantitative yield and it should be noted that this material is very readily hydrolysed and great care was required in preparing and isolating this adduct (*see Experimental*). This point is <u>not</u> obvious from the procedures in Seebach's papers but collaborators at Glaxo have encountered this problem and we are grateful for their advice on the synthesis of bicycle (**102**).

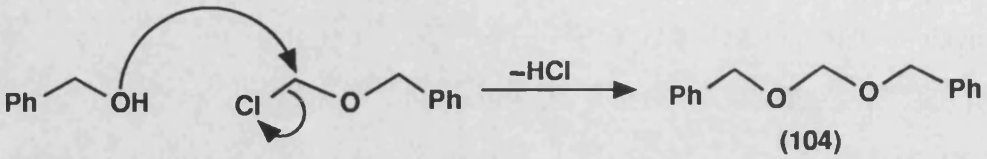
The alkylating agent required in our synthesis needed to function as an equivalent of "$^{\oplus}CH_2OH$" and benzylchloromethyl ether (**103**) can fill this role.[124] Benzylchloromethyl ether (**103**) was prepared by reacting benzyl alcohol

Scheme 52



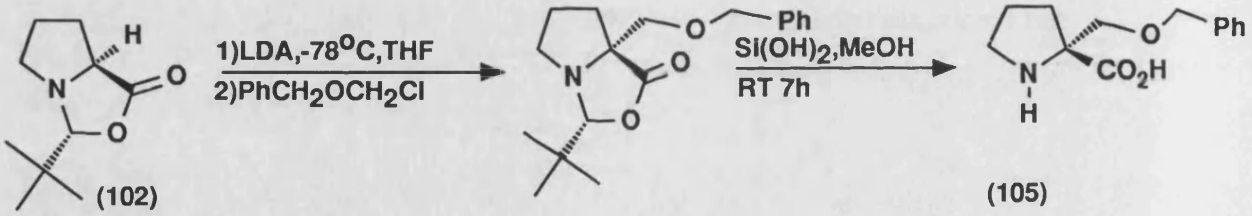
(103) (104)

Scheme 53



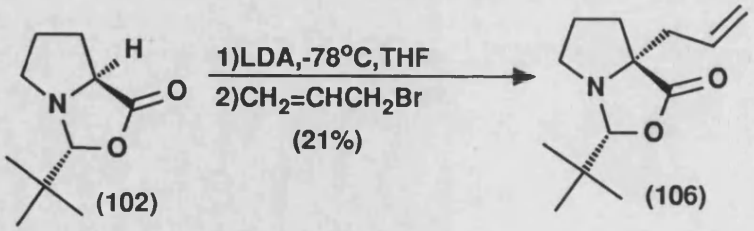
(104)

Scheme 54



(102) (105)

Scheme 55

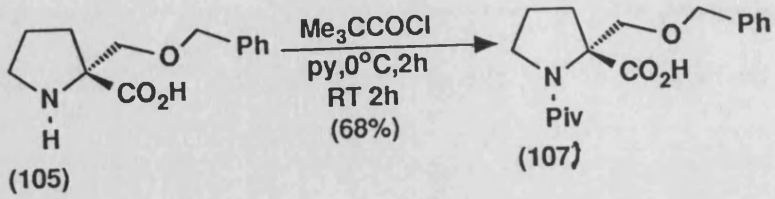


(102) (106)

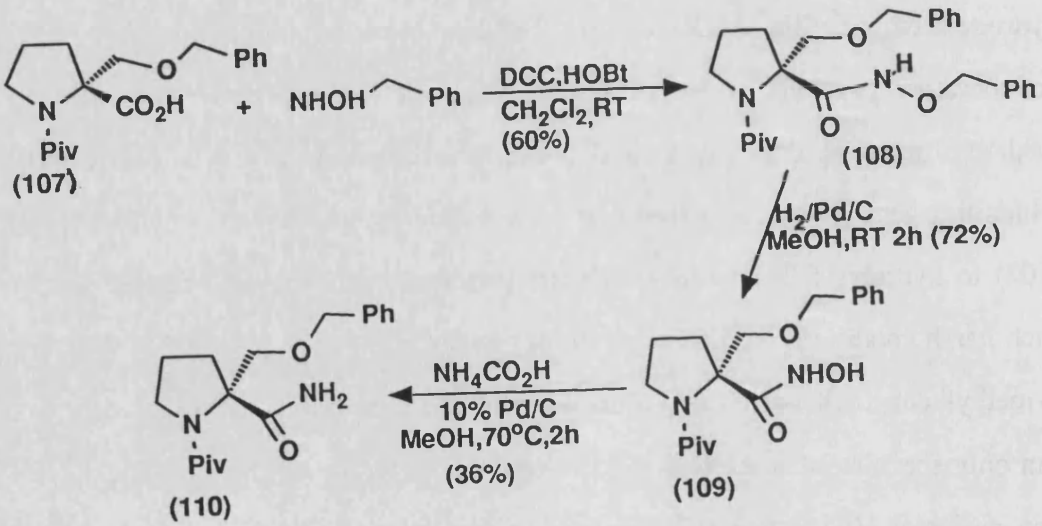
with formaldehyde under acidic condition (Scheme 52). In our hands, the yields of benzylchloromethyl ether was lower than reported in the literature [124] and significant amounts of dibenzylformal (**104**) were formed as the major by-product. This side reaction arises from reaction of benzyl alcohol with the desired product, benzylchloromethyl ether (**103**) (Scheme 53). Benzylchloromethyl ether is a toxic and hydrolytically unstable compound and upon isolation, it was often contaminated with benzyl alcohol. An alternative equivalent for "$^{\oplus}CH_2ONH_2$" is reported in *Section 5.1*.

Oxabicyclo[3.3.0]octan-4-one (**102**) was deprotonated with LDA at -78°C in THF, as described by Seebach [123] and condensed with benzylchloromethyl ether (**103**). The pivaldehyde group was reported to be removed under strongly acidic or basic conditions i.e. refluxing the α-alkylated bicycle (**102b**) in 48% hydrogen bromide (in AcOH) or by treatment with lithium amide.[123] Our collaborators (workers at Glaxo Group Research) have reported an efficient method of removal of the pivaldehyde group which involves simply stirring (**102b**) with silica gel in aqueous methanol at room temperature. Given the sensitivity of (**102**) to hydrolysis, it is somewhat surprising that Seebach was required to use such harsh conditions for this step. In our hands, an overall yield of 22% of the α-methylbenzyl ether (**105**) was obtained using these procedures (Scheme 54). We can only speculate that the low yield of α-alkylated proline obtained is due to the low purity of (**102**) and (**103**), but neither component could be purified without significant decomposition causing further complications. Another factor is that the C-2 centre in the pyrrolidine ring may be sterically too crowded for approach of benzylchloromethyl ether, we did however, repeat this alkylation reaction with allyl bromide which only gave a 21% yield of the α-allyl product (**106**), this compares to the 87% yield reported by Seebach[123] (Scheme 55). It may then be possible to optimise the yield of (**105**) but this was not attempted.

Scheme 56



Scheme 57

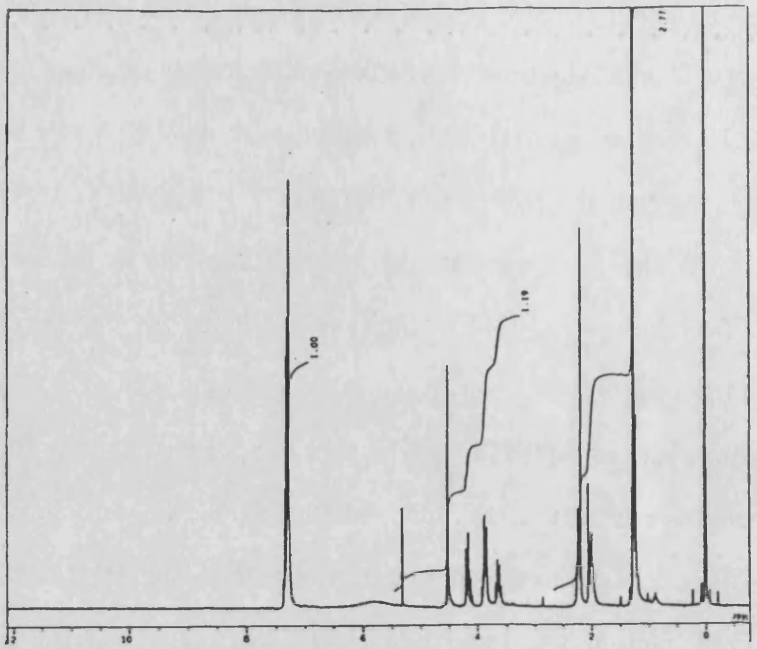


The α-alkylated proline derivative (**105**) was N-protected with pivaloyl chloride in pyridine to give (**107**) in 68% yield (Scheme 56). N-(Pivaloyl)-(2R)--methylbenzyl ether (**107**) was coupled to O-benzylhydroxylamine under standard conditions[105] with DCC and 1-hydroxybenzotriazole in dichloromethane to give (**108**) in 68% yield. Hydrogenolysis (H_2, Pd/C) served to remove only one benzyl group and we concluded that the hydroxamate O-benzyl group was the residue cleaved. This is based on the observation that an AB type signal at δ_H 4.86 + 4.94, *J* 11.2 and 11.4 Hz from compound (**108**) which corresponded for the NO$\underline{CH_2}$Ph function was absent from the ^{1}H NMR spectrum of hydroxamic acid (**109**) (Figure 10). Subjecting hydroxamic acid (**109**) to the harsher conditions of phase transfer hydrogenation (ammonium formate, 10% Pd/C, MeOH, 70°C, 2h) only led to the cleavage of the N-O bond in the hydroxamic acid function giving amide (**110**) in 36% yield. The overall reaction is outlined in Scheme 57.

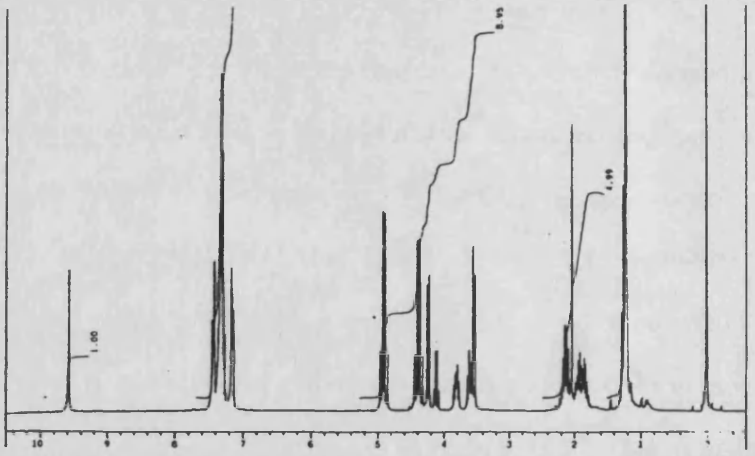
The use of boron trifluoride etherate and ethanethiol as a Hard Acid - Soft Nucleophile system has been reported to cleave alkyl/aryl benzyl ethers.[125a,b] Similarly, the use of sodium in ethanol[126] or ammonia[127] have also been reported to affect reductive cleavage of benzyl groups. However, treatment of hydroxamic acid (**109**) with boron trifluoride and ethanethiol gave a complex mixture and treatment of (**109**) with sodium in ethanol resulted in only recovery of starting material albeit in only 34% yield.

Having no success with the reductive cleavage of benzyl ether group from (**109**), we decided to reverse the order of the coupling step by first removing the benzyl group in pyrrolidine (**107**) and then couple the hydroxymethyl derivative (**111**) with O-benzylhydroxylamine. Debenzylation of compound (**107**) was achieved under atmospheric hydrogenation conditions (H_2, Pd/C) to afford the hydroxymethyl derivative (**111**) in 98% yield. The carboxylic acid function in (**111**) was then coupled with O-benzylhydroxylamine under standard peptide

Fig. 10

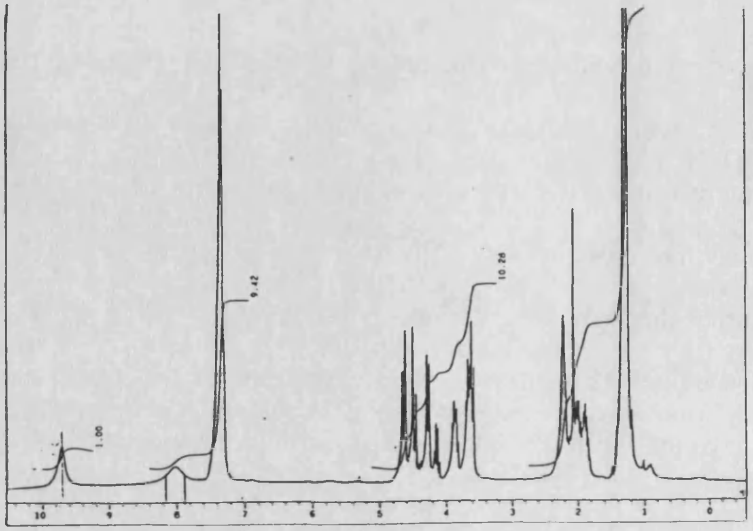


1-[N-(Pivaloyl)]-(2R)-methylbenzyl ether-(2S)-pyrrolidine carboxylic acid (107)



(108)

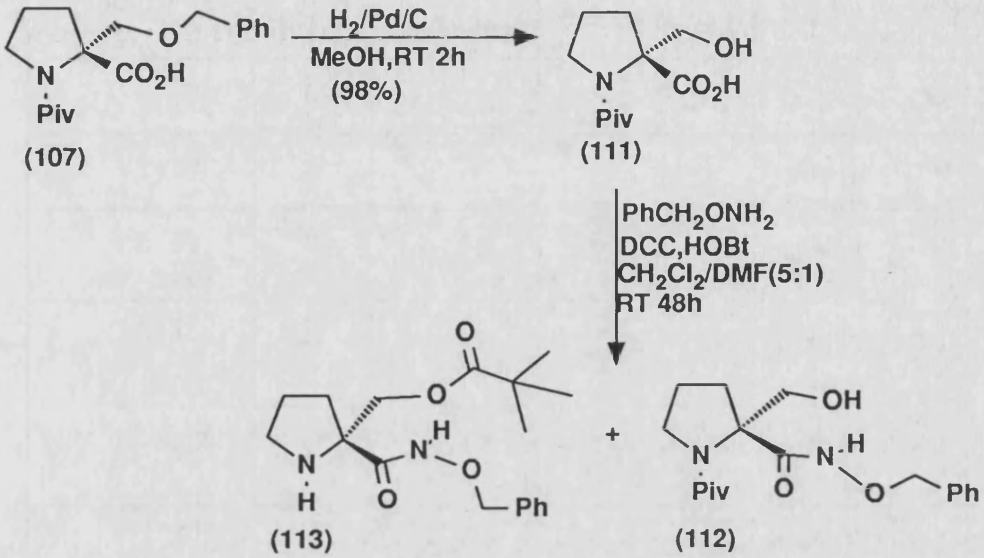
1-[N-(Pivaloyl)]-(2R)-methylbenzyl ether-N-(phenylmethoxy)-(2S)-pyrrolidine carboxamide



1-[N-(Pivaloyl)]-(2R)-methylbenzyl ether-N-(hydroxymethyl)-(2S)-pyrrolidine carboxamide
(109)

conditions[105] to give the N-(phenylmethoxy)-2-pyrrolidinecarboxamide (**112**) but this step only proceeded in low yield (10-20%) of (**112**) along with 8% of the *tert*-butyl ester (**113**), where the pivaloyl group had undergone migration to the methyleneoxy group at C-2 of the pyrrolidine ring (Scheme 58).

Scheme 58



It was apparent that the hydroxymethyl group is interfering with the coupling step and subsequent attempts directed towards improving the yield of (**112**) were unsuccessful. Efforts included using various reaction solvents such as THF, dimethylformamide and acetonitrile and varying reaction temperature from room temperature to heating at 50°C. Addition of more reactive coupling reagents, such as N,N-*bis* [2-oxo-3-oxazolidinyl]phosphinic chloride (BOP-Cl)[128] and benzotriazol-1-yloxy*tris*(dimethylamino)phosphonium hexafluorophosphate (BOP Reagent) [129] did not lead to any significant increase in the yield and the results of this aspect of the study are shown in Table 8.

[N-(Pivaloyl)]-2-(hydroxymethyl)-N-(phenylmethoxy)-2-pyrrolidine-carboxamide (**112**) was then debenzylated (H_2, Pd/C) to give 2-hydroxymethyl--N-(hydroxy)-2-pyrrolidinecarboxamide (**114**) in 65% yield (Scheme 59).

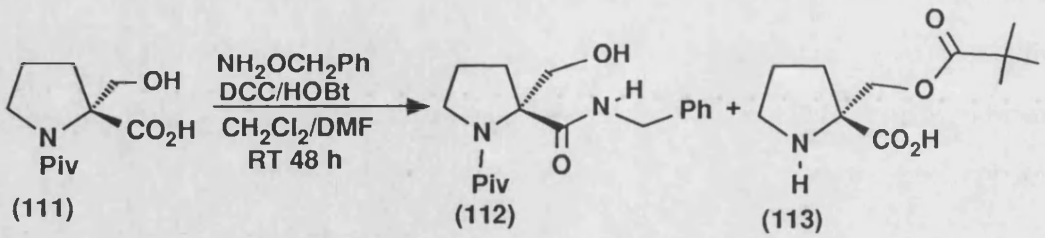


Table 8

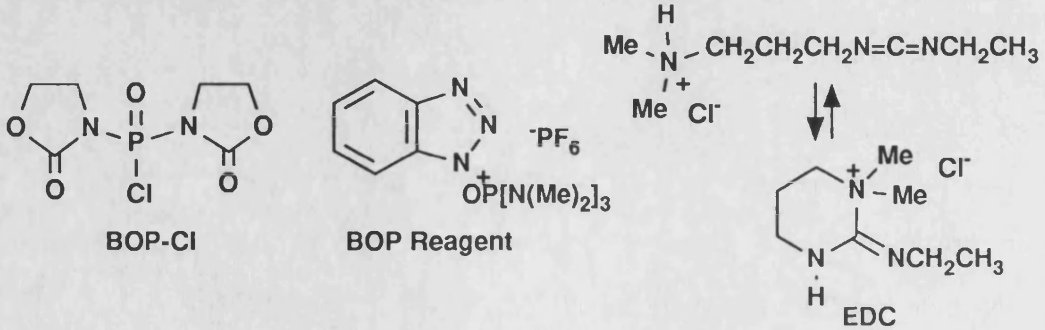
Coupling Reagent	Solvent	Temp/0°C	Rxn Time/h	Yield/% crude (112) (113)
DCC+HOBt	CH_2Cl_2	RT	20	20*
DCC+HOBt	DMF	RT	36	34(wet)
DCC+HOBt	CH_2Cl_2/DMF 5 : 1	RT	48	14* 8.5
DCC+HOBt	CH_2Cl_2DMF 6 : 1	RT	60	20 7
DCC+HOBt	CH_2Cl_2/DMF 10 : 1	RT	80	29(wet)
DCC+HOBt	CH_2Cl_2/DMF 4 : 1	RT 50	7 15	18
DCC+HOBt	CH_3CN/DMF 10 : 1	RT	20	14*
EDC+HOBt+ NMM	THF/DMF 10 : 1	RT 50	48 18	30 2 (wet)
BOP-Cl+DIEA	CH_2Cl_2	RT	20	4
BOP+DIEA	CH_2Cl_2	RT	20	28(wet)

* refers to recrystallised yield

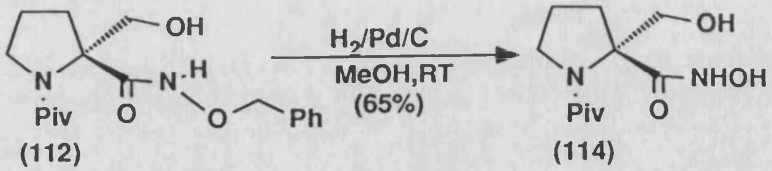
BOP-Cl = N,N-Bis[2-oxo-3-oxaolidinyl]phosphinic chloride

BOP = Benzotriazol-1-yloxytris(dimethylamino)phosphorium hexafluorophosphate

EDC = 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride



Scheme 59

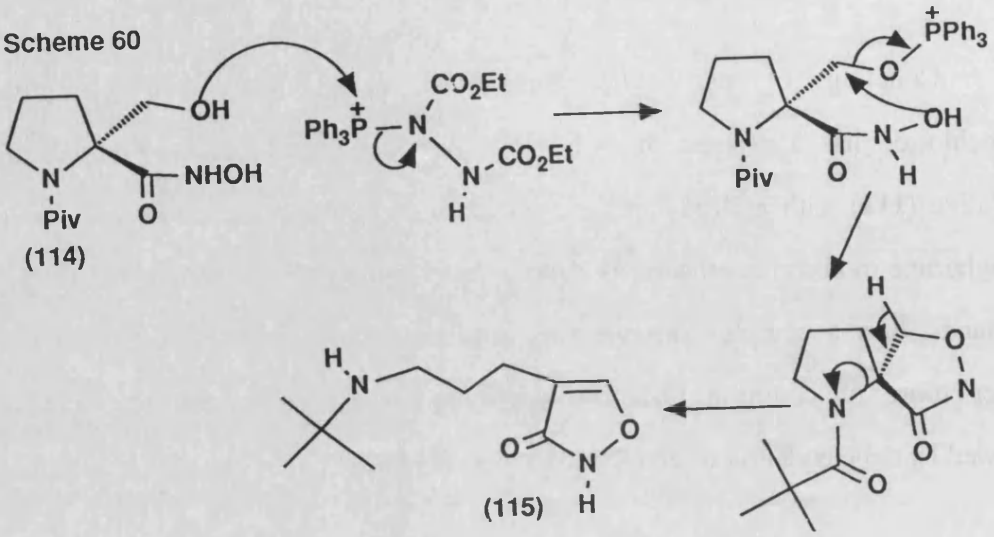


Coupling of carboxylic acids and esters with hydroxylamine hydrochloride has also been reported.[130] We sought to couple the proline derivative (**111**) with hydroxylamine hydrochloride in the presence of DCC and triethylamine in absolute ethanol as a more direct route to hydroxamate (**114**). In our hands, only a complex mixture was obtained and we decided to follow the former route of coupling O-benzylhydroxylamine to carboxylic acid (**111**), followed by debenzylation to give hydroxamate (**114**).

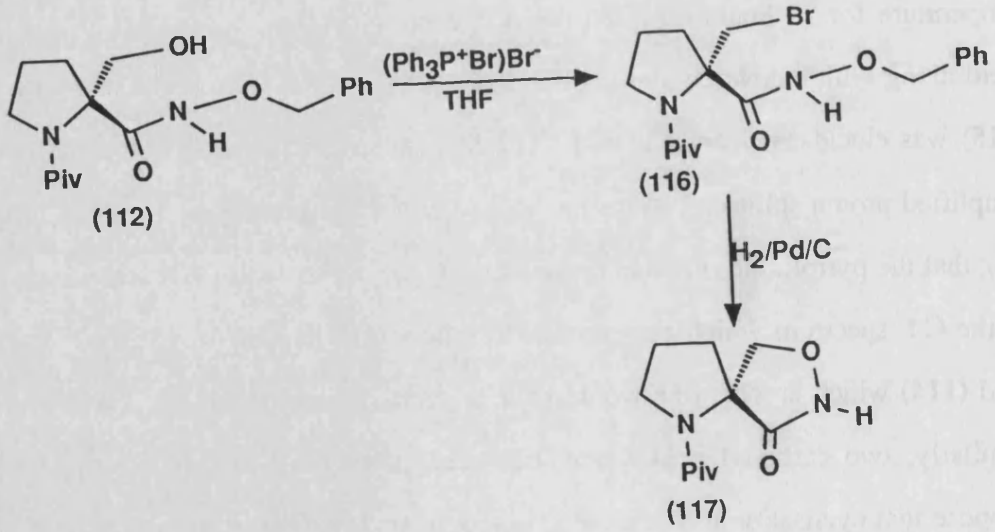
We envisaged cyclisation of hydroxamic acid (**114**) by activating the alcohol function to an alkoxyphosphonium salt by treatment of triphenylphosphine and DEAD.[116a-c] Subjecting (**114**) to this reagent combination in THF at room temperature for 20 hours afforded the ring-opened cyclised adduct (**115**) in 55% yield along with recovered starting material (**114**) in 14% yield. The structure of (**115**) was elucidated from ^{1}H and ^{13}C NMR spectra. It appears from the much simplified proton splitting pattern now observed for the protons on the pyrrolidine ring that the pyrrolidine ring had opened up. A molecular ion of 227 was observed in the C.I. spectrum which corresponded to the loss of H_2O from the hydroxamic acid (**114**) which is what one would have expected if cyclisation had taken place. Similarly, two carbonyl peaks were observed, 1740 cm^{-1} and 1630 cm^{-1}. We propose that cyclisation to form the spiro-compound had taken place, followed by fragmentation of the pyrrolidine ring (Scheme 60).

An extensive literature search on 3-hydroxyisoxazoles or 4-isoxazolidine--3-ones substituted at C-4 was undertaken. Although, mono- and di-substitution at

Scheme 60



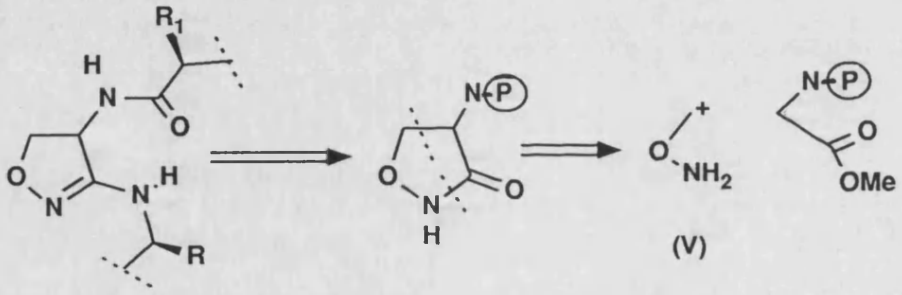
Scheme 61



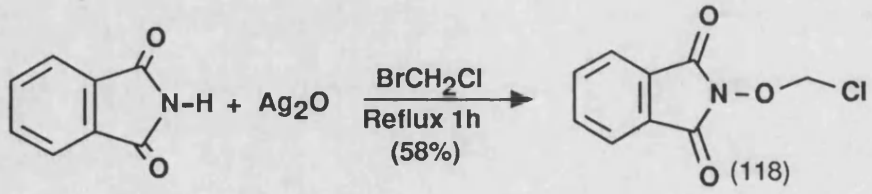
C-5 and/or at C-5 and C-4 has been reported, there was only one literature example of an alkyl substituent at C-4 of this ring system.[131] We were, however, unable to obtain any spectroscopic data for comparison with **(114)**, as only a dissertation abstract was available to us. Efforts directed towards controlling this fragmentation by reducing the reaction temperature i.e. stirring **(114)** with triphenylphosphine and DEAD in THF at 0°C for 7.5 hours followed by at -23°C for 20 hours, afforded 7% yield of the 4-isoxazoline-3-one **(115)** along with 78% yield of recovered starting material **(114)**. It is evident, that fragmentation of the putative spiro derivative is facile. Triphenylphosphine and carbon tetrabromide has been reported by Rapoport to effect ring closure of amino alcohols to give 4-substituted prolines,[132] but treatment of hydroxamic acid **(114)** with triphenylphosphine, carbon tetrabromide and diisopropylethylamine in acetonitrile only gave a complex mixture of products.

So far, the basis of our approach to cyclise hydroxamic acid **(114)** lies in the difference in reactivity between an alcohol and the hydroxyl function in the hydroxamic acid (N-OH) group. We reflected on an alternative mode of cyclisation to *spiro*-adduct **(IV)** by converting the C-2 primary alcohol of the pyrrolidine ring **(112)** to a much better leaving group, such as a mesylate or a bromide, so that upon debenzylation, ring closure can occur in a one-pot procedure (Scheme 61). Efforts to mesylate the hydroxymethyl function in carboxamide **(112)** under general mesylation conditions were unsuccessful. Similar attempts to synthesise the corresponding bromide **(116)**, by reacting carboxamide **(112)** with triphenylphosphine dibromide in THF was also unsuccessful. A new adduct was however isolated from this reaction, but we were unable to obtain any molecular ion that contained bromine. Due to the lack of starting material and available time we were unable to pursue the proposed route to *spiro*-adduct **(IV)** any further.

Scheme 62



Scheme 63



CHAPTER 5

5. *Synthesis of N-Chloromethoxyphthalimide as a Bifunctional Equivalent of NH_2-O-$CH_2^{\oplus}$*

An alternative retrosynthetic route to racemic 4-amino-3-isoxazolidinone (**1**) would be to condense an electrophilic aminoxymethylene ($NH_2OCH_2^{\oplus}$) (**V**) with a N-protected glycine methyl ester (Scheme 62). We felt that N-chloromethoxyphthalimide (**118**) would act as an experimentally viable equivalent of synthon (**V**) and although (**118**) is known in the literature, very little work has been done to exploit its synthetic potential.

N-Chloromethoxyphthalimide (**118**) was first prepared by Pratts and Gibbs[133] by heating the silver salt of the N-hydroxyphthalimide with an appropriate methyl bromide or iodide. An *in situ* preparation of (**118**) was reported by Forrester where the hydroxyphthalimide was heated in the presence of an excess of silver(I) oxide in chloroform, to give N-chloromethoxyphthalimide (**118**) in 12% yield[134] and by changing the solvent to bromochloromethane, a quantitative yield was reported. Using the procedures supplied by Professor Forrester, we were able to obtain (**118**) in 50-64% yield as a stable crystalline solid (Scheme 63).

5.12. *The Reactivity of N-Chloromethoxyphthalimide*

The reactivity of N-chloromethoxyphthalimide (**118**) towards the anions of diethyl malonate (**119**) and diethyl acetamidomalonate (**120**) was investigated. However, treating N-chloromethoxyphthalimide (**118**) with (**119**) or (**120**) under various solvent systems, bases and reactions conditions, gave none of the desired malonate adducts (Table 9 and 10). In most cases, starting material was recovered

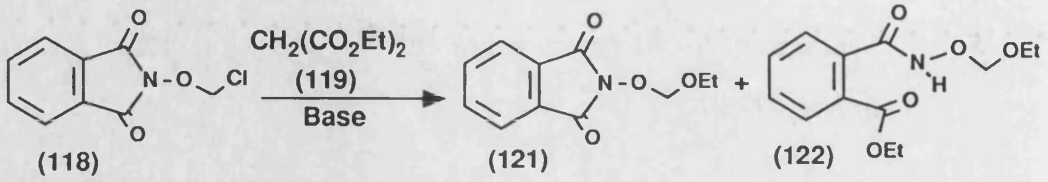
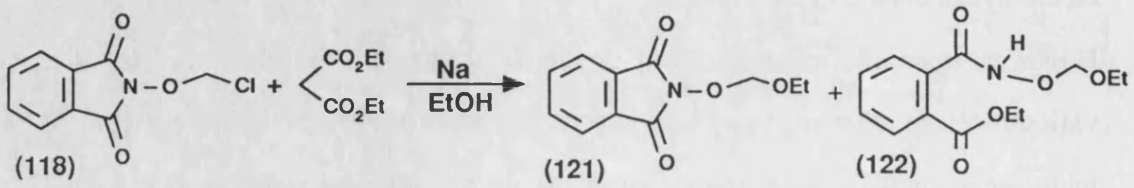


Table 9

eq./(118)	eq./(119)	eq./Base	Solvent	Rxn conditions	Yield/%crude (122) (123)
1	0.5	0.5 Na	EtOH	* (119)+Na, then add (118), stir 0°C 2h	66
1	2	2.3 Na	EtOH	* (119)+Na at -15°C, add (118). Stir -15°C 24h	27 25
1	/	1 Na	EtOH	Add (118) to soln Na in EtOH, Stir RT 3h	16 40
1	1.2	1.2 NaH	THF	* (119)+NaH, add (118), 50°C 3h	(118)***
1	1.5	1.5 NaH	THF	* (119)+NaH, add (118), stir RT 4h	(118)**
1	1.3	1.3 NaH	THF	* (119)+NaH at reflux 0.40h, then add (118), reflux 4h	(118)**
1	1.2	1.5 NaH	THF	* (119)+NaH at reflux 2h, then add (118), RT 2-5 h	(118)**

* refers to prmixing
** refers to identification by ^{1}H NMR
*** refers to identification by TLC.

Scheme 64



and identified by ^1H NMR. In the case when ethanol was used as a solvent, the ethoxide ions produced reacted with N-chloromethoxyphthalimide to give the N-ethoxymethoxy adduct (**121**), together with the ring-opened phthalimide (**122**) (Scheme 64).

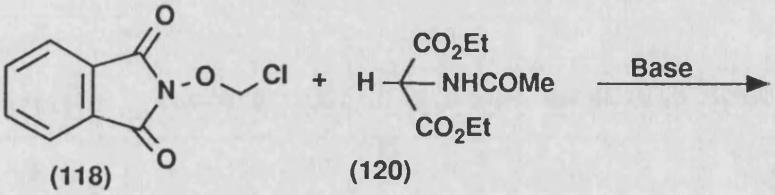


Table 10

eq./(118)	eq./(120)	eq./Base	Solvent	Rxn conditions	Results
1	1	1 LDA	THF	* (120)+LDA then add (118) at -78°C stir 0°C 20 min	(118)**
1	1.1	1.1 NaH	Benzene	* (120)+NaH at reflux 1.5 h, then add (118), reflux 24 h.	(118)**
1	1.4	1 Na	EtOH	*(120)+Na 0°C, then add (118), stir 0°C 1h, RT 16h	complex mixture
1	1	1 $^tBuO^-K^+$	THF	*(120)+base 0°C then add (118), stir 0°C 4h, RT 16 h	(118)**
1	1	1.2 NaH	THF	*(120)+NaH, add (118), stir RT 21h, reflux 8h	(118)**

* refers to premixing

** refers to identification by ^1H NMR

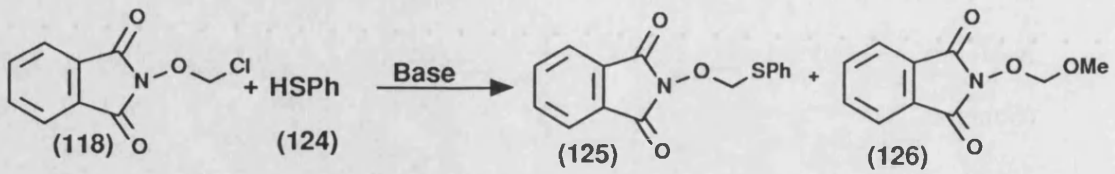
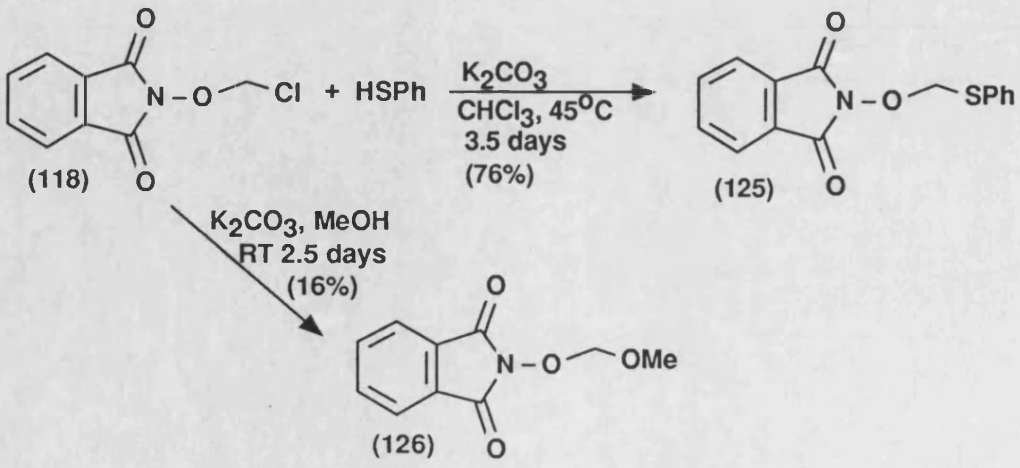


Table 11

eq./(118)	eq./(124)	eq./Base	Solvent	Rxn conditions	Results/% crude (125) (126)
1	3.5	2.5 NaH	THF	*(124)+NaH at RT, then add (118), Stir RT 28 h	49
1	2.9	3 K_2CO_3	CH_2Cl_2	*(124)+K_2CO_3 at RT, then add (118), 55°C 72h	76
1	4.2	4.1 K_2CO_3	CH_2Cl_2	*(124)+K_2CO_3 at RT 1 h, then add (118) 84 h.	79*
1	1.3	1.9 KOH	MeOH	*(124)+KOH at 0°C, then add (118), stir 0°C 6 h, RT 60h	16
1	1.3	2.1 KOH	CH_2Cl_2	*(124)+KOH at 0°C, then add (118), 0°C 6h, RT 60 h,	23

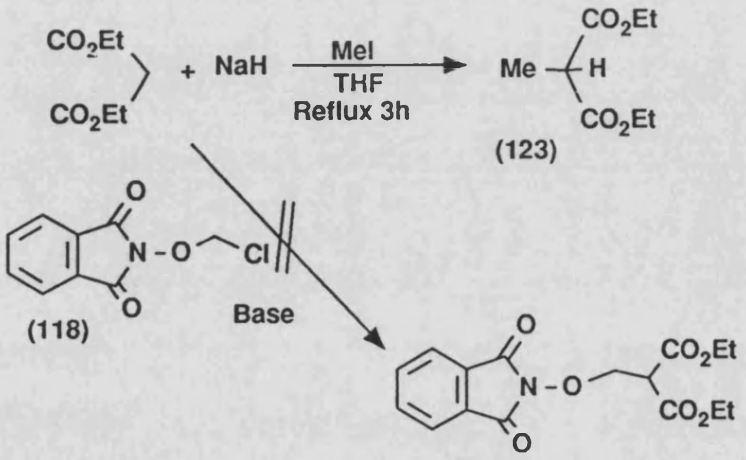
* refers to premixing
** refers to recrystallised yield

Scheme 66



To ensure that the anion of diethyl malonate was being generated, diethyl malonate was treated with sodium hydride in THF and was quenched with methyl iodide to give the alkylated adduct (**123**) in 65% yield (Scheme 65).

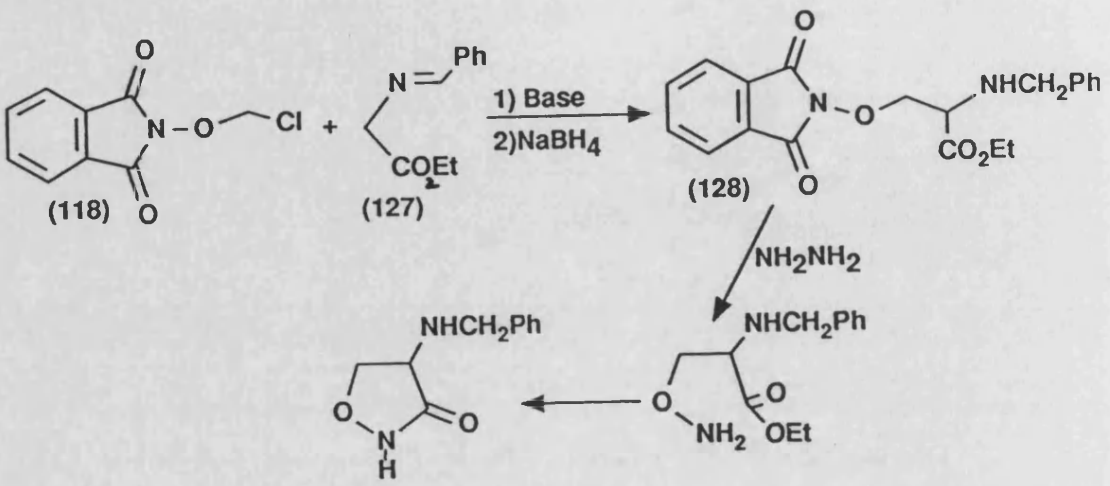
Scheme 65



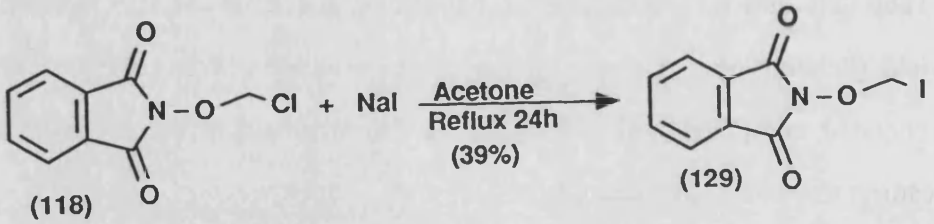
Displacement of the chloride from N-chloromethoxyphthalimide (**118**) was observed on treatment of (**118**) with thiophenol (**124**) in the presence of a base (Table 11) and the corresponding thiophenol adduct (**125**) was isolated in 76% yield (Scheme 66). Using methanol as the reaction solvent, the methoxide ions generated competed with thiophenol for the displacement of chloride in (**118**), leading to the methoxy adduct (**126**).

With these results in hand, N-chloromethoxyphthalimide (**118**) was treated with the enolate of imine (**127**) in an attempt to provide a route to the glycine adduct (**128**) which could then be cyclised to give the 4-amino-3-isoxazolidinone, once the phthalimide protecting group had been removed (Scheme 67). Adding chloride (**118**) to a premixed solution of imine (**127**) and potassium *tert*-butoxide at -78°C, as described by Stork,[139] followed by addition of sodium borohydride in ethanol at 0°C afforded only the N-benzylglycine ethyl ester, with no trace of the alkylated adduct. The lack of reactivity of chloride (**118**) towards carbon-based nucleophiles led us to synthesise

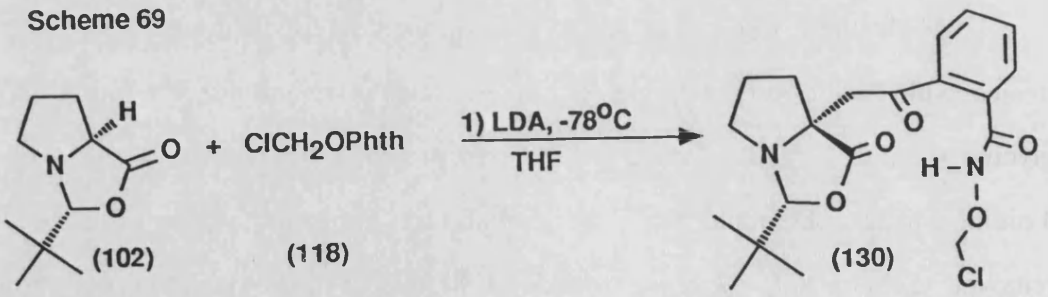
Scheme 67



Scheme 68



Scheme 69



the iodo-derivative (**129**). This was prepared in 39% yield by a halogen exchange reaction.[135] This involved heating N-chloromethoxyphthalimide in acetone with anhydrous sodium iodide (Scheme 68). Attempts to synthesise (**129**) by heating N-hydroxyphthalimide with diiodomethane in the presence of silver(I)oxide were unsuccessful. Subsequent reactions of iodomethoxyphthalimide with the enolate of diethyl malonate in THF were also unsuccessful.

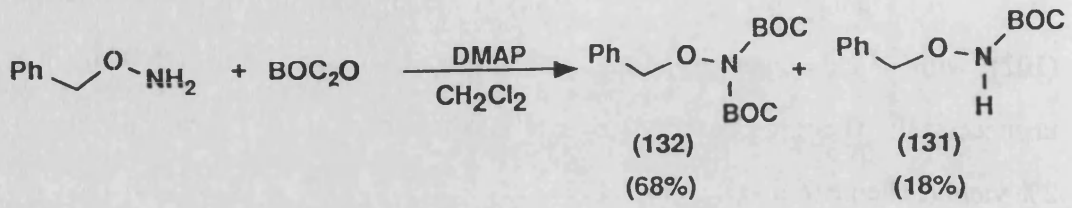
An attempt to alkylate 2-*tert*-butyl-1-aza-3-oxabicyclo[3.3.0]octan-4-one (**102**) with N-chloromethoxyphthalimide using LDA at -78°C in THF was unsuccessful. The ring-opened alkylated phthalimide adduct (**130**) was isolated in 2% yield (Scheme 69).

(Some aspects of the above work was investigated by another research student Ajmal Hussain)

5.13 Synthesis and Reactivity of N,N'-(Bis-tert-Butyloxycarbonyl)-Hydroxylamine

It is apparent that the phthalimide functionality is competing with the $-CH_2Cl$ moiety in (**118**) for nucleophiles under "S_N2-type" conditions. The reactivity of (**118**) towards silyl enol ethers of cyclohexanone under Lewis acid-mediated conditions ($TiCl_4$) were examined. The chloromethyl residue should be reactive under "S_N1-type" conditions but this study was also unproductive. An alternative strategy would be to use a different protecting group for hydroxylamine that would not have a reactive electrophilic centre and, with this in mind, the *tert*-butyloxycarbonyl (BOC) was chosen. The bulky *tert*-butyl group would sterically hinder any nucleophilic attack at the carbamate centre and its ease of removal[89d] later on in the synthesis was seen as a significant advantage. We planned to doubly protect the nitrogen in O-benzylhydroxylamine with

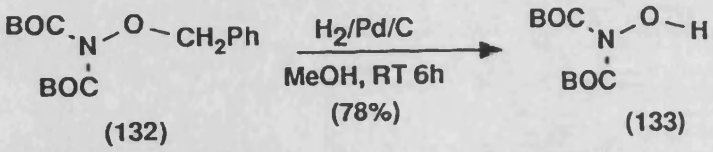
Scheme 70



(132) (131)

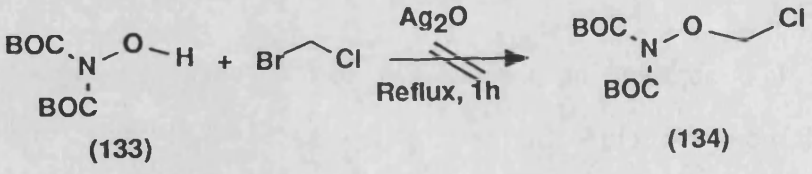
(68%) (18%)

Scheme 71



(132) (133)

Scheme 72



(133) (134)

di-*tert*-butyloxydicarbonate by following a literature procedure[136] where double N-protection of formamide with this reagent has been achieved. O-Benzyl--hydroxylamine was treated with 2.2 equivalents of BOC_2O in dichloromethane in the presence of catalytic amount of DMAP, to give the N,N′-*bis*(BOC)-O-benzyl--hydroxylamine (**132**) in 68% yield, together with the *mono*-BOC derivative (**131**) in 18% yield (Scheme 70). N,N′-*bis*(BOC)-O-benzylhydroxylamine (**132**) was N-debenzylated (H_2, 10% Pd/C) to give the corresponding N,N′-*bis*(BOC)--hydroxylamine (**133**) in 78% yield (Scheme 71).

We planned to convert N,N′-*bis*(BOC)hydroxylamine (**133**) to the chloromethoxy adduct (**134**) (Scheme 72) by using the same experimental conditions established earlier for the synthesis of N-chloromethoxyphthalimide. Upon heating hydroxylamine (**133**) in bromochloromethane in the presence of silver(I) oxide, only a complex mixture was obtained and other efforts, such as deprotonation of the hydroxylamine (**133**) with n-butyllithium at 0°C in THF followed by addition of iodochloromethane, did not give the desired product. Further investigations are required in the above reaction if N,N′-*bis*(BOC)-hydroxylamine is to provide a route to the aminoxymethylene (**V**).

EXPERIMENTAL

EXPERIMENTAL

Instrumentation and Experimental Techniques

Infrared spectra were recorded in the range 4000-600 cm^{-1} using a Perkin-Elmer 1310 grating spectrophotometer and peaks are reported (υ_{max}) in wavenumbers (cm^{-1}). The abbreviation "br" is appended to a peak to indicate significant broadening. Spectra of liquid samples were taken as thin films on sodium chloride plates, or as solutions in chloroform ($CHCl_3$). Spectra of solid samples were taken as nujol mulls.

Routine mass spectra were obtained in the electron impact (E.I.) with an ionizing potential of 70eV and in the chemical ionisation mode (C.I.), with isobutane as reagent gas. Mass spectra were also obtained in the electron impact mode with low ionizing potential (low eV E.I.) where appropriate (variable ionizing potential in the range 5-30 eV). These along with FAB and high resolution accurate mass determinations (in the E.I. mode) were recorded with a VG Analytical 7070E instrument and a VG 2000 data system. For the TOF mass spectra, these were recorded on Bioline-200 (Glaxo Group Research, Greenford). High resolution accurate mass determinations in the (C.I.) and (±FAB) mode were recorded using the S.E.R.C. facility at the University of Swansea. Where possible, the molecular ion peak is indicated along with all sizeable fragments.

Proton magnetic resonance (^1H NMR) spectra were recorded at 270 MHz, unless otherwise stated, on a Joel GNM GX FT 270 spectrometer. ^1H NMR spectra were also recorded at 60 MHz on Varian Anaspect EM-360 spectrometer, at 250 MHz on Brüker AC 250 spectrometer and at 400 MHz on a Varian VXR 400 spectrometer (Glaxo, Greenford). Carbon-13 magnetic

resonance (^{13}C NMR) spectra were recorded on a Joel GNM GX FT 270 spectrometer operating at 68 MHz, unless otherwise stated, and on the Varian VXR 400 spectrometer at Glaxo (Greenford) operating at 100.6 MHz, using 90 and 135 DEPT pulse sequences to aid in multiplicity determination.

Proton and ^{13}C NMR spectra were recorded, unless otherwise stated, in $CDCl_3$, and are expressed in parts per million (δ) downfield from internal tetramethylsilane for ^{1}H, and deuterochloroform for ^{13}C. Multiplicities are given as follows: singlet (s), doublet (d), triplet (t), quartet (q), and multiplet (m). The abbreviation "br" is appended to a multiplicity to indicate significant broadening.

^{19}F NMR spectra were recorded at 376.3 MHz on the Varian VXR 400 spectrometer at Glaxo Group Research, Greenford.

Melting points (m.p.) were determined on commercially available apparatus (Gallenkamp) and are uncorrected. Elemental microanalyses were carried out using a Carlo Erba 1106 Elemental Analyser. Optical rotations were measured using a Perkin-Elmer 141 polarimeter with concentration (c) expressed in g/100 ml.

Thin layer chromatography (TLC) was used extensively as a qualitative guide during reactions and for assessing the purity of compounds. Merck DC-alufolien Kieselgel 60 F_{254} sheets containing fluorescent indicator were used for this purpose.

Visualisation of reaction components was achieved by illumination under short wavelength (254 nm) ultraviolet light (when possible) or using a reagent (typically a 7% (w/v) solution of *dodeca*-molyphosphoric acid in methanol, or

aqueous $KMnO_4$, or ninhydrin in n-butanol) that would give a colour change with the functional groups present, as described in "Dyeing Reagents for Thin Layer and Paper Chromatography", E. Merck, Darmstadt, 1980.

Unless otherwise stated petrol refers to that fraction of petroleum spirit boiling in the range 60-80°C. Organic solvents used as eluants in chromatography were dried and distilled prior to use.

The Varian HPLC with a Brownlee Aquapore RP 300 column (7 μm) was used on an analytical scale. The Gilson HPLC with a dynamax column (packed with C-18 reversed phase silica (60 Å)) was used on a preparative scale (100 mg to 5 mg). In both cases acetonitrile (0.5% TFA) - water (0.1% TFA) was the eluant.

Medium pressure flash column chromatography were routinely employed using Kieselgel 60 (Merck 9385) (flash) and 60H silica gel (Merck 7736) for reaction component separations. A pressure gradient was developed using small, commercially available hand bellows (Gallenkamp). In all cases columns were prepared in the least polar solvent of the eluant mixture and chromatography was carried out with the least polar solvent as initial eluant, then eluting the solvent mixtures of steadily increasing polarity. Material to be chromatographed was preadsorbed onto the column support and applied as a thin layer to the top of the column. Preparative thin layer chromatography was performed using Merck 60 F_{254} silica gel, glass supported plates.

Tetrahydrofuran (THF) was pre-dried over sodium wire, then refluxed over sodium benzophenone ketyl under dry nitrogen until anhydrous. This was redistilled immediately prior to use. All reagents and solvents were purified and dried, when required, according to accepted procedures.[140]

Glassware used for water-sensitive reactions was baked in an oven at 120°C for approximately 12h and allowed to cool in a desiccator over $CaCl_2$. Flasks and stirrer bars were, however, additionally flame dried under a stream of dry nitrogen. In all experiments the excess solvent was removed with a Büchi rotary evaporator using a water aspirator at room temperature (unless otherwise stated) to avoid unnecessary decomposition. All yields quoted are of purified products and are uncorrected unless otherwise stated.

(4R)-[N-(Benzyloxycarbonyl)amino]-3-Isoxazolidinone (2)

(4R)-Cycloserine was selectively N-protected with benzyl chloroformate using Schotten-Baumann's procedures as described in the literature.[137] A crystalline solid was obtained in 63-79% yield, m.p. 138°C (from EtOAc-Petrol) (lit.,[137] m.p. 140-142°C) $[\alpha]^{19}_{D}$ + 29.66° (c 4.99 in acetone); υ_{max} (nujol mull) 3280 and 1680 cm^{-1}; δ_H (Acetone-d_6) 4.11 (1H, dd, *J* 10 and 8 Hz), 4.61-4.78 (2H, m), 5.11 (2H, s, $C\underline{H}_2Ph$), 6.82-6.90 (1H, m, 4-NH), 7.31-7.40 (5H , m, Ph), 10.30 (1H, s(br), 2-NH); m/z (C.I.) 237 (M^+ + H, 78%), 193 (18), 91 (100).

NOTE: Protection of (4R)-cycloserine using higher concentrations resulted in reaction at both nitrogen atoms leading to (**2b**).

2,4-*Bis*-[N-(benzyloxycarbonyl)amino]-3-isoxazolidinone (**2b**)

Isolated in 13% yield as colourless solid, m.p. 123-124°C (from EtOAc-Petrol) (Found: C, 61.6; H, 4.83; N, 7.60. $C_{19}H_{18}N_2O_6$ requires C, 61.62; H, 4.90; N, 7.56%); υ_{max} (nujol mull) 3350, 1765 and 1700 cm^{-1}; δ_H 4.14-4.24 (1H, m), 4.76-4.84 (2H, m), 5.16 (2H, s, $C\underline{H}_2Ph$), 5.37 (2H, s, $C\underline{H}_2Ph$), 5.65 (1H, s (br), 4-NH), 7.35-7.50 (10H, m, Phx2); m/z (+FAB) 371 (M^+ + H, 4%), 327 (5), 91 (100); (-FAB) 369 (M^+- H, 88%), 261 (59), 235 (30).

Dimer of (4R)-[*N*-(Benzyloxycarbonyl)amino]-4,5-dihydroisoxazole (5)

To an ice-cooled suspension of [N-(Cbz)]-cycloserine (**2**) (207 mg, 0.88 mmol) in acetonitrile (3 ml) was added phosphorus pentachloride (215 mg, 1 mmol). The yellow solution was stirred at 0°C for 15 min and then for 1h at room temperature. The solvent and phosphorus oxychloride were then

removed *in vacuo* to give a thick yellow oil. Purification by chromatography on silica gel using ethyl acetate-petrol as eluant gave a colourless solid (50 mg) which was then recrystallised from chloroform-petrol to give the dimer (**5**) (27 mg, 7%) m.p. 171-173°C (Found: C, 57.9; H, 12.3; N, 4.90. $C_{22}H_{22}N_4O_7$ requires C, 58.14; H, 12.33; N, 4.83%); υ_{max} ($CHCl_3$) 3470, 2880 and 1690 cm^{-1}; δ_H 4.23-4.29 (1H, m), 4.40-4.48 (2H, m), 4.75-4.90 (2H, m), 5.10-5.12 (4H, m, $\underline{CH_2}Ph$ x 2), 5.37-5.47 (2H, m), 5.63-5.70 (1H, m), 7.33-7.35 (10H, m, Ph x 2); m/z (+FAB) 455 (M^+ +H, 50%), 454 (10), 91 (100); (-FAB) 454 (M^+, 19%), 453 (67), 363 (13), 237 (7).

NOTE: Changing the solvent from MeCN to THF lead to dimer (**5**) in 47% along with the 3-chloro-(4R)-[N-(benzyloxycarbonyl)amino]-4,5-dihydro--isoxazole (**3**) in 23% yield.

Dichloro*tris*(dimethylamino)phosphorane (**4**)[81]

Hexamethylphosphorus triamide (HMPT) (3.34 ml, 0.018 mol) was added to a stirred solution of hexachloroethane (4.35 g, 0.018 mol) in acetonitrile (20 ml). The mixture was refluxed for 1h under nitrogen. The solvent and tetrachloroethene were removed *in vacuo* to give a pale yellow oil/solid. Trituration with dried petrol (30-40°C) (20 ml) gave a pale yellow solid. The petrol was then quickly decanted and the solid was washed further with dried petrol (2 x 10 ml), dried *in vacuo*, flushed with nitrogen and stored in a sealed flask to give (**4**) (3.2 g, 58%). This material was not characterised further due to its sensitivity to moisture but subsequent runs afforded the salt (**4**) in around 80% yield.

3-Chloro-(4R)-[N-(benzyloxycarbonyl)amino]-4,5-dihydroisoxazole (3)

To a stirred solution of dichloro*tris*(dimethylamino)phosphorane (12.4 g, 0.04 mol) in freshly distilled THF (50 ml) was added (2) (6.2 g, 0.026 mol). The mixture was heated at 100°C for 20h under nitrogen with vigorous stirring. After cooling, the reaction mixture was poured into water (300 ml) and extracted into ethyl acetate (4 x 100 ml), dried (Na_2SO_4), filtered and evaporated under reduced pressure to give a brown solid (10 g). Purification by chromatography on silica gel using ethyl acetate-petrol as eluant gave the chloro-adduct (3) (3.77 g, 60%), further elution gave starting material (2) (0.76 g, 12%). Chloride (3) was obtained as colourless needles m.p. 138-139°C (EtOAc-Petrol); $[\alpha]^{21}_D$ +8.75° (c 4.24 in CH_2Cl_2) (Found: C, 51.8; H, 4.32; N, 11.0. $C_{11}H_{11}ClN_2O_3$ requires C, 51.90; H, 4.35; N, 11.00%); υ_{max} (nujol mull) 3320 and 1690 cm^{-1}; δ_H 4.29 (1H, dd, *J* 10 and 6 Hz, 5-H), 4.28 (1H, t, *J* 10 Hz, 5-H), 5.15 (2H, s, $\underline{CH_2}Ph$), 5.23-5.32 (2H, m, 4-H and 4-NH), 7.36 (5H, s, Ph); δ_C 59.9 (CH), 67.6 (CH_2), 75.3 (CH_2), 128.2-128.6 (Ph_{CH}), 135.6 ($Ph_{Cquaternary}$), 155.1 ($C_{quaternary}$), 155.3 (OC=O); m/z (70eV E.I.) 256 (M^+, 0.8%), 254 (2.5), 219 (2), 91 (100); (C.I) 257 ($M^+ + H$, 3%), 255(10), 91(100).

3-Methoxy-(4R)-[N-(benzyloxycarbonyl)amino]-4,5-dihydroisoxazole (6)

To a methanol solution (2 ml) of chloride (3) (50 mg, 0.19 mmol) was added glycine methyl ester hydrochloride (27 mg, 0.2 mmol) and potassuim carbonate (34 mg, 0.25 mmol). The mixture was heated in a sealed tube at 115°C for 2h. After that time, the solvent was removed *in vacuo* to give a solid, which was dissolved in ethyl acetate (6 ml) and washed with water (2 ml). The organic extract was dried (Na_2SO_4), evaporated under reduced pressure and purified by chromatography, to afford a colourless solid. Recrystallisation from ethyl acetate-petrol afforded the 3-methoxy adduct (6)

(12 mg, 26%) as colourless needles, m.p. 97-101°C (Found: M^+ + H, 251.1032. $C_{12}H_{15}N_4O_4$ requires 251.1032); υ_{max} (nujol mull) 3290, 1675 and 1625 cm^{-1}; δ_H 3.89 (3H, s, OMe), 4.15 (1H, dd, *J* 9.5, and 6 Hz, 5-H), 4.56-4.63 (1H, m, 5-H), 5.10-5.17 (4H, m, $\underline{CH_2}$Ph, 4-H and NH), 7.36 (5H, s, Ph); δ_C 55.7 (CH), 57.9 (CH_3), 67.6 (CH_2), 75.4 (CH_2), 128.2-128.6 (Ph_{CH}), 135.8 ($Ph_{Cquaternary}$), 155.6 ($C_{quaternary}$), 166.8 (C=O); m/z (C.I.) 251 (M^+ + H, 22%), 91 (100).

3,3-Dimethoxy-(4R)-[N-benzyloxycarbonyl)amino]isoxazolidine (**6b**)

To a methanol solution (2 ml) of chloride (**3**) (43 mg, 0.17 mmol) was added potassium carbonate (34 mg, 0.25 mmol). The mixture was heated in a sealed tube at 110°C for 2h. After that time, the solvent was evaporated under reduced pressure to give a solid. Recrystallisation from ethyl acetate-petrol afforded the dimethoxy adduct (**6b**) (10 mg, 20%) as a colourless solid, m.p. 106-110°C; υ_{max}(nujol mull) 3600, 1690, 1625cm^{-1}; δ_H 3.60 (3H, s, OMe), 3.78 (3H, s, OMe), 4.01 (1H, dd, *J* 9.5 and 6.0 Hz), 4.42 (1H, t, *J* 9 Hz), 4.56 (2H, s, $\underline{CH_2}$Ph), 4.94-5.05 (1H, m), 5.32-5.38 (1H, s (br), NH), 7.23-7.25 (5H, m, Ph).

NOTE: We were unable to obtain satisfactory microanalytical data due to small amount of material available.

3-Methylamino-(4R)-[N-(benzyloxycarbonyl)amino-4,5-dihydroisoxazole (**7**)

Into a dried sealed tube was placed a solution of chloride (**3**) (102 mg, 0.4 mmol) in methanol (2 ml) and methylamine (40% w/w in water) (2 drops). The mixture was heated at 95°C for 14h, TLC, showed that reaction was incomplete so a further 2 drops of methylamine solution was added. After heating for another 7h, the solvent was removed *in vacuo* and the crude

mixture was washed with 1M aqueous sodium bicarbonate (8 ml) and extracted into dichloromethane (4 x 5 ml), dried (Na_2SO_4), filtered and evaporated under reduced pressure to give a crude solid. Recrystallisation from ethyl acetate-petrol afford (**7**) (41 mg, 44%) as a colourless solid, m.p. 168-170°C (Found: M^+ 249.1090. $C_{12}H_{15}N_3O_3$ requires 249.1112) (Found: C, 57.7; H, 6.14; N, 16.8. $C_{12}H_{15}N_3O_3$ requires C, 57.82; H, 6.07; N, 16.86%); υ_{max} (nujol mull) 3290, 1685 and 1620 cm^{-1}; δ_H 1.92-2.18 (1H, m, NHMe), 2.84 (3H, s, NHMe), 4.04 (1H, dd, *J* 9 and 5 Hz, 5-H), 4.38 (1H, t, *J* 9 Hz, 5-H), 5.02-5.09 (1H, m, 4-H), 5.09-5.18 (2H, m, CH_2Ph), 5.45-5.50 (1H, m, 4-NH), 7.35 (5H, s, Ph); m/z (70eV E.I.) 249 (M^+, 64%), 219 (27), 91 (100); (C.I.) 250 (M^+ + H, 83%) and 91 (100).

The above experimental procedure was similarly employed for the synthesis of the 3-benzylamino (**8**), 3-piperidyl (**9**) and 3-tryptamino (**10**) derivatives of (4R)-[N-(Cbz)amino]-4,5-dihydroisoxazole.

3-Benzylamino-(4R)-[N-(benzyloxycarbonyl)amino]-4,5-dihydroisoxazole (**8**)

Chloride (**3**) (102 mg, 0.4 mmol) and benzylamine (3-4 drops (excess)) in methanol (2 ml) was heated in a sealed tube at 120°C for 7 h. 3-Benzylamino (**8**) (65 mg, 50%) was isolated as a colourless solid, m.p. 161-163°C (EtOAc-Petrol) (Found: C, 66.5; H, 5.97; N, 12.7. $C_{18}H_{19}N_3O_3$ requires C, 66.52; H, 5.89; N, 12.92%); υ_{max} (nujol mull) 3300, 1680 and 1615 cm^{-1}, δ_H 2.22-2.57 (1H, s (br), NH), 4.10-4.17 (1H, m, 5-H), 4.30-4.44 (3H, m, NCH_2Ph and 5-H), 5.10 (2H, s, OCH_2Ph), 5.11-5.19 (1H, m, 4-H), 5.80 (1H, s (br), 4-NH), 7.29-7.36 (10H, m, Ph x 2); m/z (70eV E.I.) 325 (M^+, 42%), 108 (100), 106 (30), 91 (77); (C.I.) 326 (M^+ + H, 82%), 91 (100).

3-Piperidyl-(4R)-[N-(benzyloxycarbonyl)amino]-4,5-dihydroisoxazole (**9**)

Chloride (**3**) (102 mg, 0.4 mmol) and piperidine (0.08ml, 0.8 mmol) in methanol (2 ml) was heated in a sealed tube at 110°C for 18h. 3-Piperidyl (**9**) (94mg, 76%) was isolated as a colourless oil, which later solidified and was recrystallised from ethyl acetate-petrol to give (**9**) (22 mg, 18%) as colourless needles, m.p. 89-90°C (Found: M^+, 303.1575. $C_{16}H_{21}N_3O_3$ requires 303.1581) (Found: C, 63.7; H, 7.07; N, 13.8. $C_{16}H_{21}N_3O_3$ requires C, 63.35; H, 6.98; N, 13.85%); υ_{max} (nujol mull) 3300 and 1700 cm^{-1}; δ_H 1.41 (6H, s (br)), 3.08 (4H, s (br)), 4.10-4.13 (2H, m, 5-CH_2), 4.95-5.06 (3H, m, $\underline{CH_2}$Ph and 4-H), 5.36-5.42 (1H, m, 4-NH), 7.20 (5H, s, Ph); δ_C 23.9 (CH_2), 25.0 (CH_2 x 2), 47.9 (CH_2 x 2), 56.2 (CH), 67.0 (CH_2), 74.7 (CH_2), 127.9-128.4 (Ph_{CH}), 136.0 ($Ph_{Cquaternary}$), 55.2 ($C_{quaternary}$), 161.3 (OC=O); m/z (E.I.) 303 (M^+, 100%), 108 (75).

N-[3-[[3-methyl-1-(hydroxymethyl)-1-butyl]amino]-4,5-dihydroisoxazole (**11**)

Into a dried sealed tube were placed chloride (**3**) (67 mg, 0.26 mmol), (S)-leucinol (0.034 ml, 0.29 mmol) and anhydrous sodium iodide (20 mg, 0.14 mmol) in methanol (2 ml). The mixture was heated at 120°C for 24h, TLC showed that reaction was slow, so a further 0.5 equivalents of anhydrous sodium iodide (19 mg, 0.13 mmol) was added. The mixture was then heated at 120°C for another 48h. After that time, the solvent was evaporated under reduced pressure to give an oil, which was dissolved in dichloromethane (10 ml) and washed with water (3 ml). The organic extract was dried (Na_2SO_4) and evaporated under reduced pressure to give a solid. Purification by chromatography on silica gel using ethyl acetate-petrol as eluant gave starting material (**3**) (16 mg, 24%) followed by 3-methoxy derivative (**6**) (40 mg, 62%) (see above) and further elution afforded the diastereoisomeric leucinol-adduct

(**11**) (12 mg, 13%) as a yellow oil. This fraction was separated by chromatography as the major (8 mg, 9%) and the minor diastereoisomer (**11b**) (4 mg, 4%).

Major isomer (**11**): (Found: M^+, 335.1892. $C_{17}H_{25}N_3O_4$ requires 335.1845); υ_{max} ($CHCl_3$) 3420, 2930, 1705 and 1630 cm^{-1}; δ_H (400 MHz) 0.91 (6H, t, *J* 6.4 Hz, CH<u>Me$_2$</u>), 1.28-1.42 (2H, m,), 1.60-1.70 (2H, m,), 3.45 (1H, dd, *J* 11 and 5 Hz), 3.56-3.63 (1H, m,), 3.73 (1H, dd, *J* 11 and 2.2 Hz), 4.05 (1H, dd, *J* 9 and 5 Hz), 4.31-4.40 (2H, m), 5.02-5.08 (1H, m), 5.13 (2H, s, C<u>H$_2$</u>Ph), 5.73 (1H, d, *J* 7.5 Hz,), 7.37 (5H, s, Ph); δ_C 22.2 (CH_3), 22.9 (CH_3), 24.8 (CH), 39.9 (CH), 40.4 (CH_2), 53.7 (CH), 6.46 (CH_2), 67.5 (CH_2), 72.5 (CH_2), 128.2-128.6 (Ph_{CH}), 138.4 ($Ph_{Cquaternary}$), 142.7($C_{quaternary}$), 156.4 (OC=O); m/z (low 70eV E.I.) 335 (M^+, 1%), 91 (100). (C.I.) 336 (M^+ + H, 1.6%), 91 (100).

The minor diastereoisomer (**11b**) was impure and it was only characterised by ^{1}H NMR.: δ_H (400 MHz) 0.9 (6H, t, *J* 7 Hz, CH<u>Me$_2$</u>), 1.30-1.44 (2H, m), 1.59-1.66 (2H, m), 3.56-3.62 (2H, m), 3.70-3.78 (1H,m), 4.01 (1H, dd, *J* 9.5 and 4 Hz, 5-H), 4.36 (1H, t, *J* 9.5 Hz, 5-H), 4.43-4.50 (1H, m), 5.02-5.08 (1H, m,), 5.13 (2H, s, <u>CH$_2$</u>Ph), 5.26-5.30 (1H, m,), 7.32-7.39 (5H, m, Ph).

<u>Attempted preparation of 3-bromo-(4R)-[N-(benzyloxycarbonyl)amino]-</u>
<u>-4,5-dihydroisoxazole (**12**) from triphenylphosphine and carbon tetrabromide</u>
<u>in dichloromethane</u>

To an ice-cooled suspension of (4R)-[N-(Cbz)]-cycloserine (**2**) (10 g, 0.042 mol) and carbon tetrabromide (15.4 g, 0.046 mol) in dichloromethane (100 ml) was added triphenylphosphine (12.2 g, 0.046 mol) and the yellow solution was heated at 65°C for five days. After that time the mixture was poured into water (200 ml) and extracted into dichloromethane (4 x 80 ml). The combined organic extracts were dried (Na_2SO_4), filtered and evaporated under reduced

pressure to give an orange oil (36.1 g). Purification by chromatography on silica gel using ethyl acetate-petrol as eluant gave a colourless solid. Recrystallisation from ethyl acetate-petrol (40-60 v/v) gave primarily chloride (**3**) as colourless needles, m.p. 137.5-138.5°C. Halogen microanalysis revealed that there was <0.2% of bromine present in the compound. We were unable to obtain satisfactory microanalysis for the above chloride (**3**) (Found: C, 50.35; H, 4.26; N, 10.68; Br, <0.20; Cl, 13.72. $C_{11}H_{11}ClN_2O_3$ requires C, 51.87; H, 4.32; N, 11.00: Cl, 13.93%); υ_{max} (nujol mull) 3300, 1690 cm^{-1}; δ_H 4.24-4.30 (1H, m), 4.62 (1H, t, *J* 9.7 Hz), 5.10-5.20 (2H, m, $\underline{CH_2}Ph$), 5.25-5.37 (2H, m), 7.35 (5H, s, Ph); m/z (+ FAB) 301/299 ($M^+ + H$, 45%), 257 ($M^+ + H$, 35%), 255 ($M^+ + H$, 100%), 91 (100); (- FAB) 299/297 ($M^+ - H$, 52%), 255 ($M^+ - H$, 45%), 253 ($M^+ - H$, 100%).

<u>3-Bromo-(4R)-[N-(benzyloxycarbonyl)amino]-4,5-dihydroisoxazole (**12**)</u>

To an ice-cooled solution of triphenylphosphine (237 mg, 0.9 mmol) in dichloromethane (6 ml) was added dropwise bromine (0.047 ml, 0.9 mmol) and a white precipitate was formed within 5 min. The suspension was stirred at 0°C for 1h, followed by addition of [N-(Cbz)]-cycloserine (**2**) (207 mg, 0.8 mmol). The mixture was stirred at room temperature for 1.5h and then poured into 1M aqueous sodium bicarbonate (25 ml) and the product was extracted with dichloromethane (3 x 10 ml). The extracts were dried (Na_2SO_4), filtered and evaporated under reduced pressure to give a solid. Purification by chromatography on silica gel using ethyl acetate-petrol as eluant gave bromide (**12**) (44 mg, 16%). Recrystallisation from ethyl acetate-petrol gave (**12**) as colourless needles, m.p. 141-143°C (Found: C, 44.1; H, 3.62; N, 9.28. $C_{11}H_{11}BrN_2O_3$ requires C, 44.17; H, 3.71; N, 9.36%); υ_{max} (nujol mull) 3330 and 1685 cm^{-1}; δ_H 4.22 (1H, dd, *J* 10 and 6 Hz, 5-H), 4.58 (1H, t, *J* 10 Hz, 5-H), 5.17-5.20 (3H, m, $\underline{CH_2}Ph$ and NH or 4-H), 5.29-5.38 (1H, m, NH or

4-H), 7.37 (5H, s, Ph); m/z (C.I.) 301/299 (M^+ + H, 7%), 91 (100).

3-Phenylalanyl methyl ester-(4R)-[N-(benzyloxycarbonyl)amino]-
-4,5-dihydroisoxazole (**13**)

To a solution of bromide (**12**) (38 mg, 0.13 mmol) in methanol (2 ml) in a sealed tube was added (L)-phenylalanine methyl ester (57 mg, 0.26 mmol). The mixture was heated at 100°C for 10 days. After that time, the solvent was evaporated under reduced pressure to give a solid, which was redissolved in dichloromethane (5 ml) and washed with water (10 ml). The aqueous layer was then extracted with dichloromethane (3 x 8 ml) and the combined organic extracts dried (Na_2SO_4) and concentrated *in vacuo* to give an oil (67 mg). Purification by chromatography using ethyl acetate-petrol as eluant gave recovered starting material (**12**) (30 mg, 78%) followed by the (L)-phenylalanyl adduct (**13**) (7 mg, 5%) as a yellow oil (further purification by preparative TLC led to decomposition of the product): υ_{max} ($CHCl_3$) 3420, 2940 and 1720 cm^{-1}; δ_H 2.86 (1H, dd, *J* 13.5 and 7.8 Hz, $\underline{CH_2}Ph$), 3.09 (1H, dd, *J* 13.5 and 5.1 Hz, $\underline{CH_2}Ph$), 3.70-3.85 (6H, m, OMe, $\underline{CH}CO_2Me$, 5-CH_2), 3.91 (1H, dd, *J* 11.5 and 3.7 Hz, 4-H), 4.67-4.76 (1H, m, 4-NH), 5.15 (2H, s, $CO_2\underline{CH_2}Ph$), 7.18-7.39 (10H, m, Ph x 2), (1 x NH not seen); m/z (+ FAB) 398 (M^+ + H, 2%), 337 (4), 221 (20), 180 (48), 91 (100).

(4R)-[N-(9-Fluorenylmethoxycarbonyl)amino]-3-isoxazolidinone (**14**)

To an ice-cooled solution of (4R)-cycloserine (9g, 0.088 mol) in 1M aqueous sodium bicarbonate (176 ml) was added a solution of N-(9-FMOC)-
-succinimide (29.7 g, 0.088 mol) in dioxane (170 ml). On completion of addition, a white precipitate occurred and this mixture was stirred at room temperature for 2.5h. After that time, the mixture was cooled to 0°C and

acidified with concentrated hydrochloric acid to pH1. The precipitate was filtered and the solid washed with ether (5 x 120 ml). The crude solid (23.6 g) was then washed with cyclohexane (800 ml) in a Soxhlet apparatus, to give the N-protected cycloserine (**14**) (23 g, 80%) as a colourless crystalline solid, m.p. 143-145°C. Due to the solubility properties of (**14**), attempts to recrystallised (**14**) from either hot ethanol or methanol were unsuccessful; υ_{max}(nujol mull) 3313 and 1670 cm^{-1}; δ_H (DMSO-d_6) 3.94-3.97 (1H, m), 4.21-4.27 (1H, m), 4.29-4.40 (2H, m, $CO_2\underline{CH_2}$), 4.47-4.63 (2H, m), 7.30-7.47 (4H, m), 7.71 (2H, d, *J* 7.5 Hz), 7.85-7.96 (3H, m), 11.38-11.53 (1H, s(br), NH); m/z (Tof) 325 ($M^+ + H$, 9%).

3-Bromo-(4R)-[N-(9-fluorenylmethoxycarbonyl)amino]-4,5-dihydroisoxazole (**15**)

To an ice-cooled suspension of (4R)-[N-(FMOC)]-cycloserine (**14**) (2.2 g, 6.8 mmol) and carbon tetrabromide (2.5 g, 7.6 mmol) in dibromomethane (3 ml) was added triphenylphosphine (1.97 g, 7.5 mmol). The yellow solution was stirred at 0°C for 10 min and then heated between 55-60°C for 2.5h. After that time, the solvent was removed *in vacuo* to give a crude oil which was dissolved in dichloromethane (70 ml) and washed with 0.1M aqueous sodium bicarbonate solution (50 ml) and water (30 ml). The organic extract was dried (Na_2SO_4) and evaporated under reduced pressure to give an oil (4.39 g). Purification by chromatography using ethyl acetate-petrol as eluant afforded (**15**) (572 mg, 22%) as a solid. Recrystallisation from ethyl acetate-petrol gave the bromo-derivative (**15**) as a colourless needles, m.p. 198-199°C (Found: C, 56.11; H, 3.89; N, 7.29. $C_{18}H_{15}BrN_2O_3$ requires C, 55.83; H, 3.90; N, 7.23%); υ_{max} ($CHBr_3$) 3410, 3120-2920 (br), 1720 cm^{-1}; δ_H (250 MHz), 4.26-4.36 (2H, m, $CO_2\underline{CH_2}$), 4.43-4.60 (3H, m), 5.09-5.13 (1H, m, NH), 5.25-5.37 (1H, m, 4-CH), 7.33 (2H, t, *J* 7.5 Hz), 7.42 (2H, t, *J* 7.5 Hz), 7.58

(2H, d, *J* 7.5 Hz), 7.78 (2H, d, *J* 7.5 Hz); δ_C (100.6 MHz), 46.3 (CH), 61.0 (4-CH), 67.5 (5-CH_2), 72.0 (CH_2), 120.0-128.0 (Ph_{CH}), 131.8 ($Ph_{Cquaternary}$), 132.0 ($Ph_{Cquaternary}$), 139.5 ($Ph_{Cquaternary}$), 141.5 ($C_{quaternary}$), 143.7 ($C_{quaternary}$),(1 x $C_{quaternary}$ not seen); m/z (E.I.) 389 / 389 (M^+, 10%).

Reaction of 3-Bromo-(4R)-[N-(9-fluorenylmethoxycarbonyl)amino]-4,5--dihydroisoxazole (**15**) with tryptamine

To a solution of bromide (**15**) (68 mg, 0.17 mmol) in methanol (1.5 ml) was added tryptamine (40 mg, 0.19 mmol). The mixture was heated in a sealed tube at 50°C for 22h. After that time, the solvent was evaporated under reduced pressure to give an oil which was purified by chromatography using ethyl acetate-petrol to afford starting material (**15**) (23 mg, 34%) and dibenzofulvene (**16**) (18 mg, 60%), whose ^1H NMR was identical to that reported in the literature.[86]

(4R)-[N-(2,2,2-Trichloroethyloxycarbonyl)amino]-3-isoxazolidinone (**17**)

(4R)-Cycloserine (2.02 g, 0.02 mol) was selectively N-protected with 2,2,2-trichloroethyl chloroformate (2.72 ml, 0.02 mol) in 1M aqueous sodium bicarbonate (39 ml) as described for the synthesis of (4R)-[N-(FMOC)]--cycloserine (**14**). Recrystallisation from ethyl acetate afforded [N-(TROC)]--cycloserine (**17**) (2.8 g, 51%) as colourless needles, m.p. 170-171°C (Found: C, 25.7; H, 2.49; N, 10.9. $C_6H_7Cl_3N_2O_4$ requires C, 25.97; H, 2.54; N, 10.09%); υ_{max} (nujol mull) 3300, 1725 and 1670 cm^{-1}; δ_H (DMSO-d_6) 0.42 (1H, s (br), NH), 3.99 (1H, dd, *J* 10 and 8 Hz), 4.50 and 4.69 (2H, m), 4.82 (2H, s, $C\underline{H}_2CCl_3$), 8.34 (1H, d, *J* 8.4 Hz, NH); m/z (70eV E.I.) 280 (M^+, 1%), 278 (M^+, 2.3%), 277 (M^+, 0.5%), 276 (M^+, 2.3%), 101 (10); (C.I.) 281 (M^+ + H, 5%), 279 (M^+ + H, 15%), 278 (M^+ + H, 2.5%), 277 (M^+ + H, 15%), 206(5),

204 (6), 251 (3), 249 (3), 236 (11), 234 (12).

Reaction of (4R)-[N-(2,2,2-trichloroethyoxycarbonyl)amino]-3-
-isoxazolidinone (**17**) with triphenylphoshphine dibromide

To an ice-cooled solution of triphenylphosphine (229 mg, 0.87 mmol) in dibromomethane (4 ml) was added bromine (0.04 ml, 0.87 mmol) dropwise. The mixture was stirred at 0°C for 1h followed by addition of [N-(TROC)]-
-cycloserine (**17**) (212 mg, 0.76 mmol). The mixture was stirred at 0°C for 0.5h and then at room temperature for 1.5h. TLC revealed that starting material was still present, so the mixture was heated at 50-60°C for 20h. On returning, the solvent was evaporated under reduced pressure to give an oil. The oil was redissolved in ethyl acetate (10 ml) and washed with water (5 ml). The aqueous layer was then extracted with ethyl acetate (10 ml). The combined ethereal layers were dried (Na_2SO_4), filtered and evaporated under reduced pressure to give an oil (500 mg). Purification by chromatography using ethyl acetate-petrol was eluant, afforded recovered starting material (**17**) (36 mg, 17%) as the only characterisable component.

3-Chloro-(4R)-[N-(*2,2,2*-trichlorethyloxycarbonyl)amino]-4,5-
-dihydroisoxazole (**21**)

Isoxazolidinone (**17**) was converted to chloride (**21**) by heating a solution of (**17**) (986 mg, 3.5 mmol) and dichloro*tris*(dimethylamino)phosphorane (**4**) (5 equiv.) in THF (40 ml) at reflux for 20h, using the same procedure employed for 3-chloro-4-[N-(Cbz)-4,5-dihydroisoxazole (**3**). Purification by chromatography on silica gel, using ethyl acetate-petrol as eluant, gave (**21**) (81 mg, 78%) as a solid. Recrystallisation from ethyl acetate-petrol afforded (**21**) as fine colourless needles, m.p. 124-125°C (Found: C, 24.3; H, 1.90; N,

9.49. $C_6H_6Cl_4N_2O_3$ requires C, 24.35; H, 2.04; N, 9.46%); υ_{max} (nujol mull) 3300 and 1700 cm^{-1}; δ_H 4.37 (1H, dd, *J* 10 and 5.7 Hz, 5-H), 4.70 (1H, t, *J* 9.9 Hz, 5-H), 4.78 (2H, s, CH_2CCl_3), 5.32 (1H, td, *J* 9.7 and 5.9 Hz, 4-H), 5.46-5.54 (1H, m, 4-NH); δ_C 60.1 (CH), 75.2 (CH_2), 75.3 (CH_2), 149.7 ($C_{quaternary}$), 153.7 (OC=O), (1 x $\underline{C}Cl_3$ not seen); m/z (70eV E.I.) 298 (M^+, 0.5%), 296 (M^+, 1%), 294 (M^+, 1%), 261 (4.2), 259 (4.6), 163 (19), 135 (23); (C.I.) 299 (M^+ + H, 46%), 297 (M^+ + H, 100%), 295 (M^+ + H, 76%), 263 (8), 261 (15), 259 (12).

3-Bromo-(4R)-[N-(2,2,2-trichloroethyloxycarbonyl)amino]-4,5--dihydroisoxazole (**23**)

To a solution of chloride (**21**) (86 mg, 0.29 mmol) in dibromomethane (0.5 ml) was added hydrogen bromide in acetic acid (33% w/w, 0.06 ml, 0.32 mmol). The green solution was heated at 80°C for 6h and then stirred at room temperature for 20h. On returning, the solvent was removed under reduced pressure to give an oil which was dissolved in ethyl acetate (10 ml), washed with pH7 buffer (3 ml) and saturated aqueous ammonium chloride solution (3 ml). The organic extracts was dried (Na_2SO_4) and concentrated *in vacuo* to give a solid. Recrystallisation from ethyl acetate-petrol gave the bromide (**23**) (60 mg, 61%) as colourless needles, m.p. 116-117°C (Found: C, 21.3; H, 1.68; N, 8.24. $C_6H_6BrCl_3N_2O_3$ requires C, 21.16; H, 1.78; N, 8.22%); υ_{max} (nujol mull) 3300 and 1695 cm^{-1}; δ_H 4.29 (1H, dd, *J* 9.9 and 5.9 Hz, 5-H), 4.62 (1H, t, *J* 9.5 Hz, 5-H), 4.79 (2H, s, $\underline{CH}CCl_3$), 5.34 (1H, td, *J* 9.9, and 5.9 Hz, 4-H), 5.42-5.49 (1H, m, 4-NH); δ_C 62.4 (CH), 74.3 (CH_2), 74.9 (CH_2), 138.9 ($C_{quaternary}$), 153.7 (OC=O), (1 x $\underline{C}Cl_3$ not seen); m/z (C.I.) 345 (M^+ + H, 16%), 343 (55), 341 (86), 339 (48), 307 (5), 305 (10), 303 (4), 265 (5), 261 (9), 259 (4), 193 (20), 191 (21).

1-(3-Bromo-4,5-dihydro-4-isoxazolyl)-3-[2-1H-indol-3-yl)ethyl]urea (25)

To a solution of bromide (**23**) (13 mg, 0.038 mmol) in methanol (0.5 ml) in a sealed tube, was added tryptamine (10 mg, 0.065 mmol) and the mixture was heated at 100°C for 1.5h. The solvent was removed *in vacuo* to give a solid which was purified by chromatography on silica gel, using ethyl acetate-petrol as eluant, to give indolylethylamino adduct which was recrystallised from ethanol to give (**25**) (6.8 mg, 51%) as colourless needles, m.p. 184-188°C (dec.) (Found: C, 47.6; H, 4.26; N, 15.9. $C_{14}H_{15}BrN_4O_2$ requires C, 47.86; H, 4.30; N, 15.94%); υ_{max} (nujol mull) 3430, 3390, 3290 and 1625 cm^{-1}; δ_H (DMSO-d_6) 2.81 (2H, t, *J* 7 Hz, $\underline{CH_2}CH_2NH$), 3.26-3.33 (2H, m, $CH_2\underline{CH_2}NH$), 4.03 (1H, dd, *J* 9.2 and 6.7 Hz, 5-H), 4.48 (1H, dd, *J* 10.3 and 9.2 Hz, 5-H), 5.30-5.40 (1H, m, 4-H), 6.07 (1H, t, *J* 6 Hz, $CH_2CH_2\underline{NH}$), 6.83 (1H, d, *J* 9.1 Hz, 4-NH), 6.98 (1H, td, *J* 7.5 and 1.5 Hz), 7.07 (1H, td, *J* 7.5 and 1.5 Hz), 7.13 (1H, d, *J* 2 Hz), 7.33 (1H, d, *J* 7.5 Hz), 7.55 (1H, d, *J* 7.5 Hz), 10.81 (1H, s (br), NH on indole); δ_C (DMSO-d_6) 25.9 (CH_2), 40.3 (CH_2), 60.9 (CH), 73.9 (CH_2), 111.4-122.8 (Ph_{CH}), 127.3 ($Ph_{Cquaternary}$), 136.4 ($Ph_{Cquaternary}$), 142.5 ($C_{quaternary}$), 156.7 (C=O), (1 x $C_{quaternary}$ not seen); (+FAB) 353/351 (M^+ + H, 30%), 273 (3), 271 (3); (-FAB) 351/349 (M^+ - H, 78%).

[3R']-3-[3-Bromo-4,5-dihydro-4-isoxazolyl]-5-(phenylmethyl)-2,4-
-imidazolidinedinone (26)

To a solution of bromide (**23**) (88 mg, 0.26 mmol) in methanol (1 ml) in a sealed tube was added (L)-phenylalanine methyl ester (91 mg,0.51 mmol) and this mixture was heated at 100°C for 5.5 days. The solvent was removed *in vacuo* to give an orange oil which was washed with 1M aqueous sodium bicarbonate (5 ml), extracted into dichloromethane (3 x 5 ml), dried (Na_2SO_4)

and evaporated under reduced pressure. Purification of the residue by chromatography followed by preparative TLC, using ethyl acetate-petrol (1:1 v/v) as eluant, gave the imidazolidinedinone (**26**) (25 mg, 28%) as a yellow oil (Found: $M^+ + H$, 388.0140. $C_{13}H_{13}BrN_3O_3$ (Br = 79) requires 338.0140); δ_H 2.90-3.06 (1H, m, $\underline{CH_2}Ph$), 3.23-3.32 (1H, m, $\underline{CH_2}Ph$), 4.19-4.34 (1H, m, 5-H), 4.35-4.41 (1H, m, $C\underline{H}CH_2Ph$), 4.47-4.55 (1H, m, 5-H), 5.46-5.57 (1H, m, 4-H), 5.91-6.01 (1H, m, NH), 7.18-7.24 (2H, m), 7.32-7.38 (3H, m); δ_C 37.4 / 37.7 (CH_2), 57.9 / 58.1 (CH), 60.0 (CH), 70.9 (CH_2), 127.9-129.6 (Ph_{CH}), 134.2 ($Ph_{Cquaternary}$), 135.1 ($C_{quaternary}$), 154.8 (C=O), 171.4 (C=O); m/z (C.I.) 340 / 338 ($M^+ + H$, 7%), 260 (54), 91 (100).

[3R']-3-[3-Bromo-4,5-dihydro-4-isoxazolyl]-2,4-imidazolidinedinone (**27**)

To a solution of bromide (**23**) (66 mg, 0.19 mmol) in methanol (1.5 ml) in a sealed tube was added glycine methyl ester (50 mg, 0.58 mmol). The mixture was heated at 100°C for 4 days after which time the solvent was evaporated under reduced pressure and the crude solid washed with 1M aqueous sodium bicarbonate (5 ml), extracted into dichloromethane (3 x 8 ml), dried (Na_2SO_4) and concentrated *in vacuo* to give an oil. Purification by chromatography, using ethyl acetate-petrol (7:3 v/v) as eluant, gave starting material (**23**) (15 mg, 23%) and further elution afforded the imidazolidinedione adduct (**27**) (12 mg, 26%) as a colourless solid, m.p. 177-179°C (sublimed) (ethyl acetate-petrol)(Found: $M^+ + H$, 248.9671. $C_6H_7BrN_3O_3$ (Br = 79) requires 248.9671); υ_{max} ($CHCl_3$) 3440 (br), 1725 cm^{-1}; δ_H (Acetone-d_6) 4.07 (2H, s), 4.55 (1H, dd, *J* 9 and 7.7 Hz, 5-H), 4.68 (1H, dd, *J* 11.5 and 9 Hz, 5-H), 5.79 (1H, dd, *J* 11.5 and 7.9 Hz, 4-H), (1 x NH not seen); m/z (C.I.) 250 / 248 ($M^+ + H$, 100%), 168 (20), 170 (74), 143 (34).

(4R)-[N-(4-Methylphenyl)sulphonamido]-3-Isoxazolidinone (**18**)

(4R)-Cycloserine (1 g, 0.01 mol) was selectively N-protected with (4-methylphenyl)sulphonyl chloride (1.9 g, 0.01 mol) in 1M aqueous sodium bicarbonate (20 ml) as described for the preparation of [N-(FMOC)]--cycloserine (**14**). The reaction was completed after stirring at 0°C for 1h and then at room temperature for 0.45h. [N-(Tos)]-(**18**) (2 g, 79%) was isolated as a solid after recrystallisation from methanol, m.p. 201-202°C (Found: C, 46.4; H, 4.72; N, 10.5. $C_{10}H_{12}N_2O_4S$ requires C, 46.88; H, 4.72; N, 10.93%); υ_{max} (nujol mull) 3280 and 1675 (br) cm^{-1}; δ_H (DMSO-d_6) 2.39 (3H, s, PhMe), 3.67-3.73 (1H, m), 4.21-4.33 (2H, m), 7.39 (2H, d, *J* 8 Hz), 7.73 (2H, d, *J* 8 Hz), 8.41 (1H, d (br), *J* 7.5 Hz, 4-NH), (1 x NH not seen); m/z (70eV E.I.) 256 (M^+, 0.7%), 155 (47); (C.I.) 257 ($M^+ + H$, 7%), 155 (54).

Attempts to generate 3-bromo-(4R)-[N-(Tos)amino]-4,5-dihydroisoxazole (**24**) by reaction of (4R)-[N-(Tos)amino]-3-isoxazolidinone (**18**) with either Ph_3PBr_2 or Ph_3P/CBr_4 employing similar reaction conditions to those applied to (4R)-[N-(TROC)]-cycloserine (**17**), were unsuccessful.

3-Chloro-(4R)-[N-(4-methylphenyl)sulphonamido]-4,5-dihydroisoxazole (**22**)

(4R)-[N-(Tos)]-cycloserine (**18**) (189 mg, 0.7 mmol) and dichloro*tris*--(dimethylamino)phosphorane (**4**) (5 equiv.) in THF (10 ml) was heated at reflux for 20h. Following a similar workup procedure employed to isolate chloride (**3**), chloride (**22**) (160 mg, 83%) was isolated as a colourless solid after recrystallisation from ethyl acetate-petrol m.p. 129-130°C (Found: C, 43.8; H, 4.05; N, 10.25. $C_{10}H_{11}ClN_2O_3S$ requires C, 43.73; H, 4.04; N, 10.19%); υ_{max} (nujol mull) 3250 cm^{-1}; δ_H 2.46 (3H, s, PhMe), 4.22 (1H, dd, *J*

10.3 and 5.3 Hz, 5-H), 4.50 (1H, dd, *J* 10.3 and 9.3 Hz, 5-H), 4.85 (1H, td, *J* 9.4, and 5.3 Hz, 4-H), 5.04 (1H, d(br), *J* 9.3 Hz, 4-NH), 7.36 (2H, d, *J* 8 Hz), 7.78 (2H, d, *J* 8 Hz); m/z (70eV E.I) 274 (M^+, 1.5%), 276 (0.7), 155 (42), 119 (10); (C.I.) 277 ($M^+ + H$, 39%), 275 (100), 155 (74), 119 (16), 91 (94).

3-Bromo-(4R)-[N-(4-methylphenyl)sulphonamido]-4,5-dihydroisoxazole (**24**)

To a solution of chloride (**22**) (22 mg, 0.08 mmol) in dibromomethane (0.5 ml) was added hydrogen bromide in acetic acid (33% w/w, 0.016 ml, 0.08 mmol). The yellow solution was heated at 80°C for 6h and then stirred at room temperature overnight. On returning, another one equivalent of hydrogen bromide in acetic acid was added and the mixture was heated for a further hour. The solvent was evaporated under reduced pressure to give an oil which was dissolved in dichloromethane (5 ml) and washed with water (1 ml). The organic extract was dried (Na_2SO_4) and concentrated *in vacuo* and the residue recrystallised from ethyl acetate-petrol to give (**24**) (18 mg, 68%) as colourless needles m.p. 125-127°C (Found: C, 37.9; H, 3.53; N, 8.75. $C_{10}H_{11}BrN_2O_3S$ requires C, 37.63; H, 3.47; N, 8.77%); υ_{max} (nujol mull) 3260 cm^{-1}; δ_H 2.45 (3H, s, Ph$\underline{CH_3}$), 4.16 (1H, dd, *J* 10.3 and 5.3 Hz, 5-H), 4.41 (1H, t, *J* 10 Hz, 5-H), 4.86 (1H, td, *J* 9.3, and 5.3 Hz, 4-H), 5.27 (1H, d, *J* 9.5 Hz, 4-NH), 7.35 (2H, d, *J* 8 Hz), 7.78 (2H, d, *J* 8 Hz); m/z (C.I.) 321 / 319 ($M^+ + H$, 8.6%), 155 (54) and 91 (100).

3-N-Indolylethylamino-(4R)-[N-(4-methylphenyl)sulphonamido]-4,5--dihydroisoxazole (28)

A solution of bromide (24) (51 mg, 0.16 mmol) and tryptamine (55 mg, 0.34 mmol) in methanol (1.5 ml) was heated at 100°C for 2.5 days in a sealed tube. The solvent was then evaporated under reduced pressure to give a solid. Purification by chromatography using ethyl acetate-petrol as eluant to give starting material (24) (10 mg, 19%) followed by the indolylethylamino adduct (28) (8.2 mg, 12%) as a colourless solid. δ_H(Acetone-d_6) 2.45 (3H, s, PhMe), 3.03 (2H, t, *J* 7 Hz, CH_2CH_2NH), 3.35-3.45 (2H, m, CH_2CH_2NH), 3.53-3.59 (1H, s, 5-H), 3.90-3.97 (1H, m, 5-H), 4.70-4.78 (1H, m, 4-H), 5.02-5.08 (1H, m, NH), 7.02 (1H, t, *J* 7.3 Hz), 7.10 (1H, t, *J* 7.3 Hz), 7.20 (1H, s), 7.36-7.46 (3H, m), 7.62 (1H, d, *J* 7.7 Hz), 7.79 (2H, d, *J* 8.4 Hz), 10.1 (1H, s, NH on indole), (1 x NH not seen).

NOTE: A molecular ion was not found for adduct (28).

Glyoxalic acid aldoximine (35)[96]

To a cooled solution of glyoxalic acid monohydrate (6.17 g, 0.67 mol) in water (45 ml) was added hydroxylamine hydrochloride (4.69 g, 0.067 mol), followed by careful addition of sodium carbonate (7.15 g, 0.067 mol) until the solution was weakly basic. The yellow solution was left to stir at room temperature for 12h. On returning, the mixture was acidified with concentrated sulphuric acid to pH4 and then extracted into ether (500 ml) with a liquid-liquid extraction apparatus for 7h. The ether solution was evaporated under reduced pressure to give a colourless solid (5.12 g). Recrystallisation from ethyl acetate, afforded aldoxime (35) (3.36 g, 56%), m.p. 138.5-140°C (lit.[96] 139-140°C); υ_{max} (nujol mull) 3530, 3350, 3280, 1980 and 1680 (br) cm^{-1}.

Vinylphthalimide (33)[94]

Vinylphthalimide (**33**) (2.23 g, 64%) was synthesised from phthalimide (3 g, 0.02 mol) and vinyl acetate (50 ml) in the presence of sodium tetrachloropalladate (92 mg) as described in the literature.[94] The product was recrystallised from ethyl acetate-petrol to give pale yellow needles, m.p. 83.5-84.5°C (lit.,[94] 84°C), υ_{max} (nujol mull) 1715 cm^{-1}; δ_H 3.46 (1H, d, *J* 10 Hz, vinyl H), 4.50 (1H, d, *J* 16 Hz, vinyl H), 5.29 (1H, dd, *J* 16 and 9 Hz, vinyl H), 6.16 (2H, dd, *J* 5.5 and 3 Hz), 6.28 (2H, dd, J 5.5 and 3 Hz); m/z (70eV E.I.) 173 (M^+, 100%), 46 (12), 104 (45), 76 (54).

3-Bromo-5-[N-(phthaloyl)amino]-4,5-dihydroisoxazole (39)

The dibromoaldoxime was generated *in situ* by the addition of N-bromo--succimide (1.78 g, 0.01 mol) to a cooled solution of glyoxalic acid aldoxime (4.50 mg, 5.5 mmol) in dimethoxyethane (5 ml) and water (1.5 ml) as described in the literature.[93e] The dibromoaldoxime formed was added dropwise to a suspension of vinylphthalimide (1.73 g, 0.01 mol) in dimethoxyethane (5 ml) and potassium hydrogen carbonate (2 g, 0.02 mol) at room temperature. On addition, the orange solution went paler in colour and then darkened with gas (carbon dioxide) given off. After stirring for 20 min, a solid (potassium bromide) precipitated out. The mixture was left to stir at room temperature for 48h and, on returning, was filtered and washed with ethyl acetate (15 ml). The filtrate was then washed with brine (2 x 25 ml), dried ($MgSO_4$), filtered and evaporated under reduced pressure to give a light brown solid which was purified by chromatography using ethyl acetate-petrol as eluant (15:85 v/v) to give vinylphthalimide (**33**) (970 mg, 56%) and further elution (25:75 v/v) gave bromide adduct (**39**) (450 mg, 28%) as a colourless solid. Recrystallisation of bromide (**39**) from ethyl acetate-petrol afforded

colourless needles, m.p. 170-171°C (Found: C, 44.8; H, 2.32; N, 9.51. $C_{11}H_7BrN_2O_3$ requires C, 44.76; H, 2.39; N, 9.49%); υ_{max} (nujol mull) 3180, 1775 and 1715 cm^{-1}; δ_H 3.62 (2H, d, *J* 7.5 Hz, 4-CH_2), 6.55 (1H, t, *J* 7.5 Hz, 5-H), 7.79 (2H, dd, *J* 6 and 3 Hz), 7.90 (2H, dd, *J* 6 and 3 Hz); m/z (70eV E.I.) 296/294 (M^+, 4%), 215 (44). (C.I.) 297 / 295 (M^+ + H, 6%), 215 (10).

2-[2-(3-Bromo-4,5-dihydro-isoxazol-ylcarbamoyl)-benzoylamino]-3--phenylpropionic acid, methyl ester (40)

Into a dried sealed tube were placed a solution of bromide (**39**) (93 mg, 0.3 mmol) and (L)-phenylalanine methyl ester (102 mg, 0.56 mmol) in methanol (1.5 ml). The mixture was heated at 80°C for 48h and after that time the solvent was removed *in vacuo* to give an orange oil which was purified by chromatography using ethyl acetate-petrol as eluant to give starting material (**39**) (21 mg, 27%) followed by the ring-opened phthaloyl adduct (**40**) (50 mg, 35%) which was recrystallised from ethyl acetate-petrol as colourless solid, m.p. 154-155°C (Found: C, 52.9; H, 4.16; N, 8.73. $C_{21}H_{20}BrN_3O_5$ requires C, 53.16; H, 4.25; N, 8.85%); υ_{max} (nujol mull) 3280, 1750 and 1640 cm^{-1}; δ_H (DMSO-d_6) 3.02-3.11 (3H, m, C$\underline{H}_2$Ph and 4-H), 3.60-3.72 (4H, m, OMe and 4-H), 4.52-4.64 (1H, m, C$\underline{H}CO_2$Me), 6.13-6.26 (1H, m, 5-H), 7.19-7.32 (5H, m, Ph), 7.40-7.54 (4H, m, Ph), 8.82 (1H, t, *J* 7.7 Hz, NH), 9.41-9.43 (1H, m, 5-NH); m/z (+FAB) 476/474 (M^+ + H, 14%), 310 (100), 250 (75); (-FAB) 474 / 472 (M^+ - H, 83%).

3-Methanesulphonyloxy-(4R)-[N-(benzyloxycarbonyl)amino]-4,5-dihydro--isoxazole (**42**) and 2-N-methanesulphonyl-(4R)-[N-(benzyloxycarbonyl)-amino-3-Isoxazolidinone (**43**)

To an ice-cooled solution of methanesulphonyl chloride (0.04 ml, 0.52 mmol) in dichloromethane (2 ml) was added pyridine (0.042 ml, 0.52 mmol) and 4-(dimethylamino)pyridine (65 mg, 0.53 mmol). The mixture was stirred at 0°C for 10 min before additon of [N-(Cbz)]-cycloserine (**2**) (111 g, 0.47 mmol). The mixture was then stirred at room temperature for 1h, after which time the mixture was diluted with dichloromethane (20 ml) and washed with 0.1M aqueous hydrochloric acid (3 x 10 ml). The organic extract was dried (Na_2SO_4) and evaporated under reduced pressure to give a solid which was purified by chromatography, using ethyl acetate-petrol as eluant, to give the O-mesylated dihydroisoxazole (**42**) (91 mg, 62%) as colourless solid followed by the 2-N-mesylated-3-isoxazolidinone (**43**) (18 mg, 12%) as an oil.
O-Mesylate (**42**): m.p. 105-107°C (EtOAc-Petrol) (Found: C, 45.98; H, 4.48; N, 8.86; S, 9.98. $C_{12}H_{14}N_2O_6S$ requires C, 45.86; H, 4.49; N, 8.89; S, 10.18%); υ_{max} ($CHBr_3$) 3409, 3027, 2925 and 1720 cm^{-1}; δ_H (250 MHz) 3.38 (3H, s, SO_2Me), 4.37 (1H, dd, *J* 10 and 5 Hz, 5-H), 4.72 (1H, t, *J* 10 Hz, 5-H), 5.09-5.20 (2H, m, $C\underline{H}_2Ph$), 5.22-5.38 (2H, m, 4-H and NH), 7.30-7.38 (5H, m, Ph); δ_C (100.6 MHz), 39.7 (CH_3), 35.1 (4-CH), 67.8 (5-CH_2), 76.9 ($C\underline{H}_2Ph$), 128.3-128.7 (Ph_{CH}), 135.7 ($Ph_{Cquaternary}$), 155.3 ($C_{quaternary}$), 169.8 (OC=O); m/z (+FAB), 315 ($M^+ + H$, 58%), 219 (64), 91 (100).
N-Mesyl-3-isoxazolidinone (**43**): υ_{max} ($CHBr_3$) 3410, 2925, 2850 and 1765 cm^{-1}; δ_H (250 MHz), 3.28 (3H, s, SO_2Me), 4.40-4.48 (1H, m), 4.68-4.87 (2H, m), 5.13 (2H, s, $C\underline{H}_2Ph$), 5.40-5.46 (1H, s (br), NH), 7.35 (5H, s, Ph); δ_C (100.6 MHz), 40.0 (CH_3), 54.0 (4-CH), 67.8 (5-CH_2), 74.4 ($C\underline{H}_2Ph$), 128.0-131.0 (Ph_{CH}), 135.8 ($Ph_{Cquaternary}$), 169.8 (OC=O), (1 x C=O not seen).

1-[2-[-2-(1H-indol-3-yl)-ethylamino]-1-[[(methylsulphonyl)amino]oxy]-methyl]-2-oxoethyl]carbamic acid, benzyl ester (**44**) and 2-N-methane--sulphonyl-[3-Indolylethyl]amine (**45**)

To a suspension of O-mesylate (**42**) (106 mg. 0.3 mmol) in methanol (2 ml) was added tryptamine (60 mg, 0.37 mmol) and triethylamine (0.052 ml, 0.37 mmol). The mixture was stirred at room temperature for 1h, after which time the solvent was removed *in vacuo* to give an oil. The oil was dissolved in ethyl acetate (15 ml), washed with water (2 x 10 ml) and the organic extract was dried (Na_2SO_4) and evaporated under reduced pressure. The residue was purified by preparative reverse HPLC using a gradient elution of acetonitrile--water as eluant (3:7 v/v to 7:3 v/v, over a period of 30 min) gave N-methanesulphonyltryptamine (**45**) (6.2 mg, 8%) as a pink solid, followed by the ring-opened dihydroisoxazole (**44**) (11 mg, 8%) as a colourless solid. Recrystallisation of (**44**) from ethyl acetate-petrol gave a colourless solid, m.p. 134-135°C.

(**44**): (Found: C, 55.4; H, 5.49; N. 11.61; S, 6.44. $C_{22}H_{26}N_4O_6S$ requires C, 55.74; H, 5.53; N, 11.81; S, 6.75%); υ_{max} ($CHBr_3$) 3460, 3410, 2940, 1710 and 1670 cm^{-1}; δ_H (250 MHz, DMSO-d_6), 2.81 (2H, t, *J* 7.5 Hz, $C\underline{H}_2CH_2NH$), 2.99 (3H, s, SO_2Me, 3.28-3.37 (2H, m, $CH_2C\underline{H}_2NH$), 3.95-4.12 (2H, m), 4.30-4.40 (1H, m), 5.02 (1H, d, *J* 12.5 Hz, part of AB system), 5.09 (1H, d, *J* 12.5 Hz, part of AB system), 6.94-7.16 (3H, m), 7.30-7.40 (6H, m, Ph and NH), 7.53 (2H, t, *J* 7.5 Hz), 8.12-8.17 (1H, m, $CH_2CH_2\underline{NH}$), 9.95 (1H, s, $\underline{NH}SO_2Me$), 10.81 (1H, s, NH on indole); δ_C (100.6 MHz, DMSO-d_6) 24.9 (CH_2), 36.1 (CH_3), 39.8 (CH_2), 53.6 (CH), 65.5 (CH_2), 75.7 ($C\underline{H}_2Ph$), 111.1-128.1 (Ph_{CH}), 111.5 ($Ph_{Cquaternary}$), 126.9 ($Ph_{Cquaternary}$), 136.1 ($Ph_{Cquaternary}$), 136.7 ($Ph_{Cquaternary}$), 155.6 (C=O), 168.5 (OC=O); m/z (+ FAB), 475 (M^+ + H, 51%), 315 (3), 288 (7), 143 (85), 91 (60).

(45): (Found; M^+, 238.0776. $C_{11}H_{14}N_2O_2S$ requires 238.0776); υ_{max} ($CHBr_3$) 3460, 2970, 2820 cm^{-1}; δ_H (250 MHz, DMSO-d_6), 2.84-2.93 (5H, m, $SO_2\underline{Me}$, $C\underline{H_2}CH_2NH$), 3.22 (2H, m, $CH_2C\underline{H_2}NH$), 6.96-7.11 (3H, m), 7.20 (1H, s), 7.34 (1H, d, *J* 7.5 Hz), 7.53 (1H, d, *J* 7.5 Hz), 10.84 (1H, s, NH on indole); δ_C (Methanol-d_4), 24.8 (CH_2), 40.2 (SO_2Me), 42.3 (CH_2), 112.58 (Ph_{CH}), 113.1 ($Ph_{Cquaternary}$), 119.5-124.1 (Ph_{CH}), (2 x $Ph_{Cquaternary}$ not seen); m/z (Tof) 238 (M^+, 20%).

NOTE: Repeating the above reaction in dimethylformamide solution afforded (45) in 35% and (44) in 10% yield.

Reaction of 2-N-methanesulphonyl-(4R)-[N-(benzyloxycarbonyl)amino]--3-isoxazolidinone (43) with Tryptamine

Compound (44) was similarly isolated as a colourless solid (66 mg, 53%) from the reaction of the 2-N-mesylate (43) (82 mg, 0.26mmol) with tryptamine (48 mg, 0.29 mmol) in triethylamine (0.04 ml, 0.29 mmol) and methanol (2 ml) at room temperature for 2h.

1-[3-[-2-(1H-indol-3-yl)-ethylamino]-2-[(trifluoromethyl)sulphonyl]-3--[[(trifluoromethyl)sulphonyloxy]-4-isoxazolyl]carbamic acid, phenyl ester (46b)

To a solution of (4R)-[N-(Cbz)]-cycloserine (100 mg, 0.42 mmol) and 2,6-di--*tert*-butyl-4-methylpyridine (101 mg, 0.49 mmol) in dichloromethane (5 ml) at -78°C was added dropwise triflic anhydride (0.08 ml, 0.47 mmol). After stirring at -78°C for 10 min, TLC showed loss of starting material. Tryptamine (160 mg, 1.0 mmol) was then added and the mixture was further stirred at -78°C for another 45 min and then at 0°C for 1h. On returning, the mixture was poured into water (10 ml), extracted with dichloromethane (3 x

10 ml) and the extracts were dried (Na_2SO_4) and evaporated under reduced pressure to give an orange oil/solid. Purification by chromatography using ethyl acetate-petrol as eluant afforded the *bis*-trifluoromethanesulphonyl derivative (**46b**) (124 mg, 44%); υ_{max} ($CHBr_3$) 3464, 3360, 2923, 1725, 1509, 1231, 1200 cm^{-1}; δ_H (250 MHz, DMSO-d_6), 2.95 (2H, t, *J* 7.5 Hz, $C\underline{H}_2CH_2NH$), 3.41 (2H, t, *J* 7.5 Hz, $CH_2C\underline{H}_2NH$), 4.43 (1H, dd, *J* 10 and 6.25 Hz, 5-H), 4.82 (1H, t, *J* 10 Hz, 5-H), 5.08 (2H, s, $C\underline{H}_2Ph$), 5.35-5.46 (1H, m, 4-H), 6.96-7.11 (2H, m), 7.21 (1H, d, *J* 2.5 Hz), 7.30-7.40 (6H, m), 7.50 (1H, d, *J* 7.5 Hz), 8.40 (1H, d, *J* 7.5 Hz, NH), 9.33 (1H, s,), 10.90 (1H, s, NH on indole); δ_C (100.6 MHz), 26.4 (CH_2), 44.4 (CH_2), 54.4 (CH), 68.0 (CH_2), 77.4 (CH_2), 111.2 ($Ph_{Cquaternary}$), 111.8-129.0 (Ph_{CH}), 127.0 ($Ph_{Cquaternary}$), 135.5 ($Ph_{Cquaternary}$), 136.5 ($Ph_{Cquaternary}$), 159.0 ($C_{quaternary}$), (2 x $\underline{C}F_3$ and 1 x $C_{quaternary}$ not seen); δ_F (376.3 MHz, DMSO-d_6) -97.2 and -92.3; (+FAB) 661 (M^+ + H, 0.75%), 660 (8), 369 (10), 292 (58) 219 (10); (-FAB) 659 (M^+ - H, 1.6%), 367 (17), 291 (28), 149 (100).

NOTE: An accurate molecular ion was not found for this compound.

[3'R]-[3-[[2-(3-indolyl)ethyl]amino]-4,5-dihydroisoxazolylcarbamic acid, phenylmethyl ester (**10**)

To a solution of chloride (**3**) (5.12 g, 0.02 ml) in methanol (30 ml) in a sealed tube was added tryptamine (6.33 g, 0.04 mmol). The orange solution was heated at 100°C for 48h, after which time the solvent was removed *in vacuo* to give a solid. Purification by chromatography using ethyl acetate-petrol as eluant afforded starting material (**3**) (1.52 g, 30%) followed by the 3-methoxy adduct (**6**) (0.34 g, 6.8%) and then finally the indolylethylamino adduct (**10**) (3.62 g, 48%). Recrystallisation from ethyl acetate-petrol gave (**10**) as a pale yellow solid, m.p. 171-173°C; $[\alpha]^{19}_D$ + 44.7° (c 4.0 in acetone) (Found: C, 66.4; H, 5.86; N, 14.8. $C_{21}H_{22}N_4O_3$ requires C,66.65; H,5.86; N,14.80%);

υ_{max} (nujol mull) 3425, 3310, 3220, 1700 and 1640 cm^{-1}; δ_H (250 MHz, DMSO-d_6) 2.91 (2H, t, *J* 7.5 Hz, $C\underline{H}_2CH_2NH$, 3.28 (2H, m, $CH_2C\underline{H}_2NH$), 3.78 (1H, t, *J* 8 Hz, 5-H), 4.21 (1H, t, *J* 8.7 Hz, 5-H), 4.91-5.11 (3H, m, 4-H and $\underline{CH}_2Ph$), 6.03 (1H, t, *J* 5 Hz, $CH_2CH_2\underline{NH}$), 6.98 (1H, td, *J* 8 and 1.1 Hz), 7.07 (1H, td, *J* 8 and 1.1 Hz), 7.13 (1H, d, *J* 2.2 Hz), 7.32-7.40 (6H, m), 7.54 (1H, d, *J* 7.8 Hz), 7.99 (1H, d, *J* 8.4 Hz, 4-NH), 10.81 (1H, s, NH on indole),; δ_C (Acetone-d_6) 25.5 (CH_2), 48.9 (CH_2), 59.8 (CH), 67.1 (CH_2), 72.7 (CH_2), 112.2-129.3 (Ph_{CH}), 113.4 ($Ph_{Cquaternary}$), 137.7 ($Ph_{Cquaternary}$), 159.7 ($C_{quaternary}$), (2 x $Ph_{Cquaternary}$ and 1 x $C_{quaternary}$ not seen); m/z (+FAB) 379 (M^+ + H, 89%), 91 (78); (-FAB) 377 (M^+ - H, 21%), 269 (100).

3-N-[3-[[2-(3-indolyl)ethyl]amino]-(4)-amino-3-isoxazolidine (**47**)

To a solution of indolylethylamino derivative (**10**) (1.74 g, 0.004 mmol) in methanol (50 ml) was added 10% palladium over charcoal catalyst (0.35 g) and the mixture was then subjected to atmospheric hydrogenation for 20h. After that time, the catalyst was removed by filtration through celite and the filtrate was evaporated under reduced pressure to give the isoxazolidine (**47**) (1.10g, 97%) as a yellow foam; υ_{max} ($CHCl_3$) 3470, 3400, 2890 and 1610 cm^{-1}; δ_H (methanol-d_4) 3.03 (2H, t, *J* 7.5 Hz, $C\underline{H}_2CH_2NH$), 3.27-3.32 (2H, m, $CH_2C\underline{H}_2NH$), 3.41-3.61 (4H, m), 5.85 (1H, m, $CH_2CH_2N\underline{H}$), 6.95-7.15 (3H, m), 7.32 (1H, d, *J* 7.5 Hz), 7.56 (1H, d, *J* 7.5 Hz), 11.85 (1H, s (br), NH on the indole), (3 x NH not seen); m/z (+FAB) 247 (M^+ + H, 100%), 231 (3), 161 (2).

[3'R]-[3-[[2-(3-indolyl)ethyl]amino]-4,5-dihydro-4-isoxazolylamine (48)

To a solution of indolylethylamino derivative (**10**) (520 mg, 1.4 mmol) in dichloromethane (4 ml) was added anisole (0.3 ml, 2.7 mmol), followed by dropwise addition of trifluoromethanesulphonic acid (0.86 ml, 9.6 mmol) with vigorous stirring. The mixture was diluted with dichloromethane (5 ml) and washed with 1M aqueous hydrochloric acid (15 ml). The acidified extract was cooled to 0°C and made alkaline with 5M aqueous sodium hydroxide to pH 8-9. The mixture was extracted with dichloromethane (2 x 50 ml), dried (Na_2SO_4) and evaporated under reduced pressure to give a yellow oil. Purification by chromatography, using dichloromethane-methanol (9:1 v/v) as eluant, gave the corresponding free amine (48) (211 mg, 62%) as an oil, $[\alpha]^{20}_D$ - 10.9° (c 10.75 in CH_2Cl_2); υ_{max} ($CHCl_3$) 3480, 3300, 2920 and 1610 cm^{-1}; δ_H (DMSO-d_6) 1.98 (2H, s (br), NH_2), 2.94 (2H, t, *J* 7.5 Hz, $C\underline{H}_2CH_2NH$), 3.25-3.29 (2H, m, $CH_2C\underline{H}_2NH$), 3.52 (1H, t, *J* 9 Hz), 4.07-4.22 (2H, m), 5.84 (1H, t, *J* 6 Hz, $CH_2CH_2\underline{NH}$), 6.98 (1H, td, *J* 7.5 and 1.5 Hz), 7.07 (1H, td, *J* 7.5 and 1.5 Hz), 7.17 (1H, d, *J* 2.2 Hz), 7.34 (1H, d, *J* 8 Hz), 7.55 (1H, d, *J* 7.7 Hz), 10.82 (1H, s, NH on indole); δ_C 24.3 (CH_2), 43.5 (CH), 59.5 (CH_2), 73.8 (CH_2), 111.2-122.6 (Ph_{CH}), 136.1 ($Ph_{Cquaternary}$), 165.4 ($C_{quaternary}$), (2 x $Ph_{Cquaternary}$ not seen); m/z (70eV E.I.) 244 (M, 9%), 128 (8), 77 (31). (C.I.) 245 ((M^+ + H, 100%).

[3'R]-[2-[[3-[[2-(3-indolyl)ethyl]amino]-4,5-dihydro-4-isoxazolylamino]--1-methyl-2-oxoethyl]carbamic acid, phenylmethyl ester (49)

To an ice-cooled solution of N-(Cbz)-(L)-alanine (74 mg, 0.33 mmol) in dimethylformamide (1 ml) was added 1-hydroxybenzotriazole hydrate (69 mg, 0.5 mmol) and dicyclohexylcarbodiimide (73 mg, 0.35 mmol). After stirring for 5 min, a white precipitate formed, and to this was added a solution of

3-tryptamino-(4R)-amino-4,5-dihydroisoxazole (**48**) (50 mg, 0.2 mmol) in dimethylformamide (0.5 ml). The mixture was stirred at room temperature for 20h after which time the dicyclohexylurea was filtered off and washed with ethyl acetate (10 ml). The filtrate was evaporated under reduced pressure to give a yellow oil which was purified by chromatography, using ethyl acetate-petrol (8:2 v/v) as eluant, to give the tripeptide (**49**) (69 mg, 80%) as a pale yellow solid, m.p. 155.5-157°C (EtOAc); $[\alpha]^{21}_D$ + 65.0° (c 0.52 in ethanol) (Found: C, 64.1; H, 5.94; N, 15.2. $C_{24}H_{26}N_5O_4$ requires C, 64.13; H, 6.06; N, 15.57%); υ_{max} ($CHCl_3$) 3420 (br), 2920, 1715, 1670 and 1620 cm^{-1}; δ_H (DMSO-d_6) 1.20 (3H, d, *J* 7.5 Hz, CHMe), 2.89-2.96 (2H, m, $C\underline{H}_2CH_2NH$), 3.21-3.30 (2H, m, $CH_2C\underline{H}_2NH$), 3.79 (1H, dd, *J* 9 and 6.7 Hz, 5-H), 4.04-4.09 (1H, m), 4.19 (1H, t, *J* 9 Hz, 5-H), 5.00 (1H, d, *J* 13.1 Hz, part of AB system), 5.05 (1H, d, *J* 12.5 Hz, part of AB system), 5.11-5.20 (1H, m), 5.83 (1H, t, *J* 5.2 Hz, $CH_2CH_2\underline{NH}$), 6.96 (1H, td, *J* 8 and 1.1 Hz), 7.06 (1H, td, *J* 8.2 Hz and 1.1 Hz), 7.31-7.37 (6H, m,), 7.47 (1H, d, *J* 7.1 Hz), 7.53 (1H, d, *J* 7.8 Hz), 8.66 (1H, d, *J* 7.9 Hz), 10.30 (1H, s, NH on indole); m/z (+FAB) 450 (M^+ + H, 80%), 289 (4), 91 (89), 77 (23); (-FAB) 448 (M^+ - H, 19%), 342 (17), 242 (8).

[3'R]-N-[2-[[3-[[2-(3-indolyl)ethyl]amino]-4,5-dihydro-4-isoxazolylamino]--1-methyl-oxoethyl]amine (**50**)

To a dichloromethane solution (3 ml) of tripeptide (**49**) (248 mg, 0.55 mmol) was added anisole (0.12 ml, 1 mmol), followed by dropwise addition of trifluoromethanesulphonic acid (0.29 ml, 3 mmol). The mixture was stirred at room temperature for 5 min and then cooled to 0°C and neutralised with 5M aqueous NaOH solution. The product was then extracted into dichloromethane (6 x 10 ml) and dried (Na_2SO_4) and concentrated *in vacuo* to give the tripeptide (**50**) (150 g, 86%) as a yellow solid. Recrystallisation from ethanol afforded (**50**) as a colourless solid, m.p. 158-159°C $[\alpha]^{21}_D$ +116.7° (c

0.45 in ethanol) (Found: C, 60.9; H, 6.79; N, 21.6. $C_{16}H_{21}N_5O_2$ requires C, 60.94; H, 6.71; N, 22.20%); υ_{max} ($CHCl_3$) 3480, 3340, 2920, 2850, 1650 and 1620 cm^{-1}; δ_H (DMSO-d_6) 1.10 (3H, d, *J* 7.0 Hz, CHMe), 1.60-2.00 (1H, s (br), NH), 2.93 (2H, t, *J* 7.5 Hz, CH_2CH_2NH), 3.22-3.30 (3H, m, CH_2CH_2NH and CHMe), 3.80 (1H, dd, *J* 8.8 and 6.4 Hz, 5-H), 4.18 (1H, t, *J* 8.6 Hz, 5-H), 5.10 (1H, dd, *J* 8.6 and 6.4 Hz, 4-H), 5.96 (1H, t, *J* 5.6 Hz, CH_2CH_2NH), 6.97 (1H, td, *J* 7.9 Hz and 1.3 Hz), 7.06 (1H, td, *J* 8.2 Hz and 1.2 Hz), 7.12 (1H, d, *J* 2.4 Hz), 7.34 (1H, d, *J* 7.9 Hz), 7.55 (1H, d, *J* 7.7 Hz), 10.30 (1H, s, NH on indole), (2 x NH not seen); m/z (C.I.) 316 (M^+ + H, 1.6%), 129 (16), 91 (41).

[3'R]-N-[2-[2-[[3-[[2-(3-indolyl)amino]-4,5-dihydro-4-isoxazolylamino]--1-methyl-2-oxoethyl]amino]-1-methyl-2-oxoethyl]carbamic acid, phenylmethyl ester (**51**)

The coupling of tripeptide (**50**) (55 mg, 0.16 mmol) with N-(Cbz)-(L)-alanine (59 mg, 0.26 mmol) was performed using the same procedures as described for the synthesis of tripeptide compound (**49**). The crude solid was purified by chromatography using ethyl acetate-petrol as eluant to give tetrapeptide (**51**) (57 mg, 69%) as a pale yellow solid, m.p. 208-210°C (dec.)(ethanol-ether); $[\alpha]^{21}_D$ +30.25° (c 4.0 in ethanol) (Found: C, 62.3; H, 6.28; N, 15.7. $C_{27}H_{32}N_6O_5$ requires C, 62.29; H, 6.20; N, 16.14%); υ_{max} (nujol mull) 3270, 1680 and 1620 cm^{-1}; δ_H (DMSO-d_6) 1.18-1.23 (6H, m, CHMe x 2), 2.89-2.96 (2H, m, CH_2CH_2NH), 3.22-3.30 (2H, m, CH_2CH_2NH), 3.77 (1H, dd, *J* 8.6 and 6.6 Hz, 5-H), 4.07 (1H, t, *J* 7.1 Hz), 4.16-4.24 (1H, m, 5-H), 4.26 (1H, t, *J* 7.3 Hz), 4.97 (1H,d, *J* 12.6 Hz, part of AB system), 5.04 (1H, d, J 12.6 Hz, part of AB system), 5.10-5.19 (1H, m, 4-H), 5.90 (1H, t, *J* 5.3 Hz, CH_2CH_2NH), 6.96 (1H, td, *J* 7.5 and 1.1 Hz), 7.06 (1H, td, *J* 8.2 and 1.3 Hz), 7.14 (1H, d, *J* 2.2 Hz), 7.30-7.37 (6H, m,), 7.53 (2H, t, *J* 8.4 Hz), 8.00 (1H, d, *J* 7 Hz, NH), 8.59 (1H, d, *J* 7 Hz, NH), 10.3 (1H, s, NH on indole); m/z (+FAB) 521 (M^+ + H,

84%), 342 (9), 178 (4), 91 (24); (-FAB) 519 (M^+ - H, 10%), 411 (100), 385 (10).

[3'R]-N-[2-[[3-(3-indolyl)ethyl]amino]-4,5-dihydro-4-isoxazolylyamino]--1-methyl-2-oxoethyl]benzamide (52)

Tripeptide (**50**) (39 mg, 0.12 mmol) was coupled to benzoic acid (26 mg, 0.21 mmol) in the presence of 1-hydroxybenzotriazole hydrate (39 mg, 0.29 mmol) and dicyclohexylcarbodiimide (47 mg, 0.22 mmol) in dimethylformamide (1 ml) as described for the synthesis of tripeptide (**49**). The crude oil was purified by chromatography on silica gel using ethyl acetate-petrol as eluant to afford the N-benzoyl-tripeptide (**52**) (43 mg, 85%) as a pale yellow solid, m.p. 196-197°C (ethanol-ether), $[\alpha]^{19}_D$ +91.95° (c 0.27 in CH_2Cl_2) (Found: C, 66.0; H, 6.09; N, 16.6. $C_{23}H_{25}N_5O_3$ requires C, 65.86; H, 6.00; N, 16.69%); υ_{max} ($CHCl_3$) 3490, 3250, 2990, 1720, 1630 and 1620 cm^{-1}; δ (DMSO-d_6) 1.34 (3H, d, *J* 7 Hz, CHMe), 2.94-2.99 (2H, m, CH_2CH_2NH), 3.25-3.30 (2H, m, CH_2CH_2NH), 3.77 (1H, dd, *J* 8.4 and 7.3 Hz, 5-H), 4.24 (1H, t, *J* 8.8 Hz, 5-H), 4.40-4.45 (1H, m, 4-H), 5.22 (1H, q, *J* 7.3 Hz, CHMe), 5.90 (1H, t, *J* 5.5 Hz, CH_2CH_2NH), 6.95 (1H, td, *J* 7 and 1.4 Hz), 7.08 (1H, td, *J* 7 Hz and 1.4 Hz), 7.17 (1H, d, *J* 2.2 Hz), 7.33 (1H, d, *J* 8.1 Hz), 7.42-7.58 (4H, m), 7.90 (2H, d, *J* 7 Hz), 8.58 (1H, d, *J* 6.6 Hz, NH), 8.75 (1H, d, *J* 8.1 Hz, 5-NH), 10.8 (1H, s, NH on indole); m/z (70eV E.I.) 419 (M^+, 0.6%), 105 (100), 77 (56).

(2S)-[N-(Benzyloxycarbonyl)amino]-3-Isoxazolidinone (53)

(4S)-Cycloserine (1.98 g, 0.19 mol) was N-protected with benzyl chloroformate (3.05 ml, 0.021 mol) as described in the literature[137] for the R-isomer. The N-protected product (**53**) (5.15 g, 74%) was isolated as a crystalline solid, m.p. 134-5-135.5°C (from EtOAc-petrol) $[\alpha]^{19}_D$ -29.74° (c

5.08 in acetone); υ_{max} (nujol mull) 3280 and 1670 cm^{-1}; δ_H 4.10 (1H, dd, *J* 11 and 7 Hz), 4.63-4.72 (1H, m), 4.80 (1H, t, *J* 8 Hz), 5.12 (2H, s, $\underline{CH_2}Ph$), 5.27 (1H, s(br), NH), 7.36 (5H, s, Ph), (1 x NH not seen).

NOTE: The 2,4-*Bis*-[N-(Cbz)amino]-3-isoxazolidinone (1.03 g, 14%) was also isolated and it was only identified by TLC by co-running the compound with the corresponding R-isomer.

3-Chloro-(4S)-[N-(benzyloxycarbonyl)amino]-4,5-dihydroisoxazole (54)

(4S)-[N-(Cbz)]-cycloserine (**53**) (5.09 g, 0.021 mol) was heated with five equivalents of dichloro*tris*(dimethylamino)phosphorane (**4**) in THF (60 ml), as described for the (R)-isomer (**2**). The reaction was completed after heating for 2.5h and the chloro adduct (**54**) (4.58 g, 86%) was isolated as colourless needles, m.p. 136.5-137.5°C (ethyl acetate-petrol), $[\alpha]^{18}_D$ -8.96° (c 4.85 in CH_2Cl_2) (Found: C, 51.6; H, 4.27; N, 11.0. $C_{11}H_{11}ClN_2O_3$ requires C, 51.88; H, 4.35; N, 10.99%); υ_{max} (nujol mull) 3400 and 1680 cm^{-1}; δ_H 4.29 (1H, dd, *J* 10 and 6 Hz, 5-H), 4.69 (1H, t, *J* 10 and 5 Hz, 5-H), 5.10-5.20 (2H, m, $C\underline{H_2}Ph$), 5.25-5.35 (2H, m, 4-H and NH), 7.36 (5H, s, Ph); m/z (70eV E.I.) 254 (M^+, 2.5%), 91 (100). (C.I.) 255 (M^+ + H, 10%), 257 (3), 219 (2.5), 108 (10), 91 (100).

[3'S]-[3-[[2-(3-indolyl)ethyl]amino]-4,5-dihydroisoxazolylcarbamic acid, phenylmethyl ester (55) and 3-methoxy-(4S)-[N-(benzyloxycarbonyl)amino]--4,5-dihydroisoxazole (55b)

Chloride (**54**) (4.10 g, 0.16 mmol) in methanol (15 ml) was heated with tryptamine (5.18 g, 0.032 mol) in a sealed tube, at 100°C for 20h. The solvent was removed under reduced pressure to give a solid which was purified by chromatography using ethyl acetate-petrol as eluant to give starting material

54) (1.4 g, 34%), followed by the 3-methoxy (**55b**) (0.35 g, 9%) and finally the indolylethylamino adduct (**55**) (3.15g, 52%) as a straw coloured solid, m.p. 168-170°C (EtOH), $[\alpha]^{19}_{D}$ -42.5° (c 3.87 in acetone) (Found: C, 66.7; H, 5.88; N, 15.0. $C_{21}H_{22}N_4O_3$ requires C, 66.68; H, 5.86; N, 14.80%); υ_{max} (nujol mull) 3380, 3260, 3200, 1675 and 1615 cm^{-1}; δ_H (DMSO-d_6) 2.92 (2H, t, *J* 7.5 Hz, $C\underline{H}_2CH_2NH$), 3.22-3.31 (2H, m, $CH_2C\underline{H}_2NH$), 3.79 (1H, dd, *J* 9 and 7.5 Hz, 5-H), 4.20 (1H, t, *J* 9 Hz, 5-H), 4.92-5.01 (1H, m, 4-H), 5.02 (1H, d, *J* 12 Hz, part of AB ystem), 5.09 (1H, d, *J* 12 Hz, part of AB system), 6.05 (1H, t, *J* 6 Hz, $CH_2CH_2\underline{NH}$), 6.98 (1H, t, *J* 7.5 Hz), 7.07 (1H, t, *J* 7.5 Hz), 7.17 (1H, d, *J* 2.2 Hz), 7.30-7.43 (5H, m, Ph), 7.55 (1H, d, *J* 7.7 Hz), 8.02 (1H, d, *J* 8.4 Hz), 10.8 (1H, s, NH on indole), (1 x NH not seen); m/z (C.I.) 379 (M^+ + H, 0.9%) and 245 (1).

3-Methoxy adduct (**55b**) was isolated as a colourless solid: m.p. 117.9-118°C (EtOAc-Petrol) (Found: M^+ + H, 251.1032. $C_{12}H_{15}N_2O_4$ requires 251.1032); υ_{max} (nujol mull) 3260, 1670 and 1615 cm^{-1}; δ_H 3.89 (3H, s, OMe), 4.16 (1H, dd, *J* 9.8 and 6 Hz, 5-H), 4.56-4.63 (1H, m, 5-H), 5.10-5.21 (4H, m, $C\underline{H}_2Ph$, 4-H and NH), 7.36 (5H, s, Ph); δ_C 55.7 (CH), 57.9 (CH_3), 67.4 (CH_2), 75.4 (CH_2), 128.2-128.5 (Ph_{CH}), 135.4 ($Ph_{Cquaternary}$), 155.5 ($C_{quaternary}$), 167.3 (C=O); m/z (70eV E.I.) 250 (M^+, 0.8%), 106 (20), 91 (100. (C.I.) 251 (M^+ + H, 28%), 219 (0.8), 106 (18), 91 (100).

[3'S]-[3-[[2-(3-indolyl)ethyl]amino]-4,5-dihydro-4-isoxazolylamine (**56**)

Dihydroisoxazole (**55**) (1.02 g, 0.027 mol) was debenzylated to the corresponding amino derivative (**56**) using the same procedure employed for the conversion of (4R)-(**10**) to (**48**). The product was purified by chromatography on silica gel using dichloromethane-methanol (9:1 v/v) as eluant to give (**56**) (464 mg, 70%) as a colourless oil, $[\alpha]^{20}_{D}$ +12.51° (c 8.4 in CH_2Cl_2); δ_H (DMSO-d_6) 2.94 (2H, t, *J* 7.3 Hz, $C\underline{H}_2CH_2NH$), 3.24-3.37 (2H,

m, $CH_2\underline{CH_2}NH$), 3.55 (2H, m), 4.10-4.23 (2H, m), 5.87 (1H, m, $CH_2CH_2\underline{NH}$), 6.80 (1H, td, *J* 7.7 and 1.1 Hz), 7.07 (1H, td, *J* 7.0 and 1.1 Hz), 7.18 (1H, d, *J* 2.2 Hz), 7.35 (1H, d, *J* 8.1 Hz), 7.55 (1H, d, *J* 7.9 Hz), 10.2 (1H, s, NH on indole), (1 x NH not seen).

[3'S]-N-[2-[[3-[[2-(3-indolyl)ethyl]amino]-4,5-dihydro-4-isoxazolylamino]--1-methyl-2-oxoethyl]carbamic acid, phenylmethyl ester (**57**)

Indolylethylamino derivative (**56**) (0.8 g, 3.3 mmol) was coupled to N-(Cbz)-L-alanine (1.13 g, 5 mmol) as described for the conversion of (4R)-(**48**) to (**49**). Purification by chromatography, using ethyl acetate-petrol as eluant, afforded tripeptide (**57**) (1.14 g, 77%) as a pale yellow solid. m.p. 197-199°C (dec.), $[\alpha]^{19}_D$ -5.0° (c 0.52 in DMF) (Found: C, 64.1; H, 6.02; N, 15.4. $C_{24}H_{26}N_5O_4$ requires C, 64.13; H, 6.06; N, 15.40%); υ_{max} (nujol mull) 3350, 3270, 1680, 1645 and 1620 cm^{-1}; δ_H (DMSO-d_6) 1.19 (3H, d, *J* 7 Hz, CH<u>Me</u>), 2.88-2.96 (2H, m, $\underline{CH_2}CH_2NH$), 3.23-3.32 (2H, m, $CH_2\underline{CH_2}NH$), 3.78-3.84 (1H, m), 4.04 (1H, t, *J* 7.5 Hz), 4.16 (1H, t, *J* 9 Hz), 4.96-5.12 (3H, m, $\underline{CH_2}Ph$ and 4-H), 5.94 (1H, t, *J* 5.3 Hz, $CH_2CH_2\underline{NH}$), 6.97 (1H, td, *J* 8.1 and 1.1 Hz), 7.07 (1H, td, *J* 8.2 and 1.2 Hz), 7.16 (1H, d, *J* 2.2 Hz), 7.30-7.38 (5H, m, Ph), 7.46 (1H, d, *J* 7.3 Hz), 7.54 (1H, d, *J* 7.5 Hz), 8.46 (1H, d, *J* 7.7 Hz, NH), 10.3 (1H, s, NH on indole); m/z (+FAB) 450 (M^+ + H, 74%), 91 (63). (-FAB) 448 (M^+ - H, 10%), 314 (7), 151 (33), 135 (27).

[3'S]-N-[2-[[3-[[2-(3-indolyl)amino]-4,5-dihydro-4-is oxazolylamino]--1-methyl-2-oxoethyl]amine (**58**)

Tripeptide (**57**) (282 mg, 0.62 mmol) was N-debenzylated to (**58**) using the same procedure employed for the conversion of the (4R)-(**49**) to (**50**). The product was purified by chromatography using dichloromethane-methanol (9:1

v/v) as eluant, to afford amine (58) (124 mg, 63%) as a colourless oil, $[\alpha]^{20}_D$ -61.8° (c 0.5 in ethanol); υ_{max} 3470, 3380-3320 (br), 2920, 1665 and 1635 cm^{-1}; δ_H (DMSO-d_6) 1.80 (3H, d, *J* 7 Hz, CHMe), 2.95 (2H, t, *J* 7.3 Hz, CH_2CH_2NH), 3.22-3.30 (2H, m, CH_2CH_2NH), 3.40-3.48 (2H, m), 3.82 (1H, dd, *J* 8.8 and 5.5 Hz), 4.19 (2H, t, *J* 8.8 Hz),, 5.09 (1H, dd, *J* 9 and 6 Hz), 6.10 (1H, t, *J* 5.5 Hz, CH_2CH_2NH), 6.97 (1H, td, *J* 7.7 and 1.1 Hz), 7.08 (1H, td, *J* 7 and 1.1 Hz), 7.15 (1H, d, *J* 2.2 Hz), 7.32 (1H, d, *J* 8 Hz), 7.53 (1H, d, *J* 7.7 Hz), 8.04-8.08 (1H, s (br), NH), 10.82 (1H, s, NH on indole); m/z (+FAB) 316 (M^+ + H, 100%), 228 (14); (-FAB) 314 (M^+ - H, 77%), 299 (6), 272 (13).

[3'S]-N-[2-[2-[[3-[[2-(3-indolyl)ethyl]amino]-4,5-dihydro-4-isoxazolylamino]--1-methyl-2-oxoethyl]amino]-1-methyl-2-oxoethyl]carbamic acid, phenylmethyl ester (59)

Tripeptide (58) (51 mg, 0.16 mmol) was coupled to N-(Cbz)-L-alanine (59 mg, 0.27 mmol) using the procedure employed for the conversion of (4R)-(48) to (49). Care was required to wash the product thoroughly with methanol from the dicyclohexylurea precipitate. Purification by chromatography using ethyl acetate-petrol (7:3 v/v) followed by dichloromethane-methanol (9:1 v/v) as eluant gave the tetrapeptide (59) (41 mg, 48%). m.p. 248-249°C (dec.),$[\alpha]^{19}_D$ -29.39° (c 0.33 in DMF) (Found: M^+ + H, 521.2512. $C_{27}H_{33}N_6O_5$ requires 521.2512); υ_{max} (nujol mull) 3390, 3320, 3260, 1690 and 1640 (br) cm^{-1}; δ_H (DMSO-d_6), 1.19 (3H, d, *J* 7.1 Hz, CHMe), 1.20 (3H, d, *J* 7.0 Hz, CHMe), 2.88-2.97 (2H, m, CH_2CH_2NH), 3.22-3.33 (2H, m, CH_2CH_2NH), 3.80 (1H, dd, *J* 8.8 and 5.5 Hz, 5-H), 4.05 (1H, t, *J* 7.3 Hz), 4.16 (1H, t, *J* 8.8 Hz, 5-H), 4.24 (1H, t, *J* 7.1 Hz), 4.97-5.12 (3H, m, CH_2Ph and 4-H), 5.94 (1H, t, *J* 5.3 Hz, CH_2CH_2NH), 6.97 (1H, td, *J* 7.9 and 1.1 Hz), 7.06 (1H, td, *J* 8 and 1.2 Hz), 7.16 (1H, d, *J* 2.4 Hz), 7.30-7.37 (6H, m, Ph and NH), 7.48 (1H, d, *J* 7.4 Hz), 7.55 (1H, d, *J* 7.5 Hz), 7.98 (1H, d, *J* 7.3 Hz, NH), 8.55 (1H, d, *J* 8

Hz, NH), 10.3 (1H, s (br) NH on indole), (1 x NH not seen); δ_C (100.6 MHz, DMSO-d_6) 18.6 and 18.7 (CH_3 x 2), 24.9 (CH_2), 44.4 (CH_2), 47.8 (CH), 50.6 (CH), 56.9 (CH), 65.9 (CH_2), 72.7 (CH_2), 111.9-128.9 (Ph_{CH}), 136.8 ($Ph_{Cquaternary}$), 137.5 ($Ph_{Cquaternary}$), 156.3 ($C_{quaternary}$), 159.2 (OC=O), 172.7 (NC=O), 173.0 (NC=O), (2 x $Ph_{Cquaternary}$ not seen); m/z (+FAB) 521 (M^+ + H, 35%), 314 (0.7), 206 (1.4), 149 (100), 91 (48); (-FAB) 519 (M^+ - H, 20%), 385 (8), 227 (7).

[3'S]-N-[2-[[3-[[2-(3-indolyl)ethyl]amino]-4,5-dihydro-4-isoxazolylamino]--1-methyl-2-oxoethyl]benzamide (**60**)

Tripeptide (**58**) (112 mg, 0.36 mmol) was coupled with benzoic acid (76 mg,0.61 mmol) following the procedure described for the conversion of (4R)-(**50**) to (**52**). The mixture was purified by chromatography, using ethyl acetate-petrol as the eluant, to afford the product N-benzoyl-tripeptide (**60**) (91 mg, 61%) as a pale yellow solid which we were unable to recrystallise. m.p. 246.5-248°C (dec.), $[\alpha]^{20}_D$ -42.86° (c 0.14 in CH_3CN) (Found: M^+ + H, 420.2036. $C_{23}H_{26}N_5O_3$ requires 420.2035); υ_{max} (nujol mull) 3390, 3350, 3280 and 1645 cm^{-1}; δ_H (DMSO-d_6) 1.37 (3H, d, *J* 7.3 Hz, CHMe), 2.83-2.89 (2H, m, CH_2CH_2NH), 3.21-3.32 (2H, m, CH_2CH_2NH), 3.85 (1H, dd, *J* 8.4 and 6.6 Hz), 4.18 (1H, t, *J* 8.4 Hz), 4.40-4.52 (1H, m), 5.15 (1H, m) 5.84 (1H, t, *J* 6 Hz, CH_2CH_2NH), 6.98 (1H, t, *J* 7.7 Hz), 7.07 (1H, t, *J* 7.3 Hz), 7.16 (1H, d, *J* 1.5 Hz), 7.33 (1H, d, *J* 8 Hz), 7.45-7.58 (4H, m), 7.91 (2H, d, *J* 7 Hz), 8.52 (1H, d, *J* 6.6 Hz), 8.67 (1H, d, *J* 8.1 Hz), 10.82 (1H, d, NH on indole); δ_C (100.6 MHz, DMSO-d_6) 18.3 (CH_3), 25.0 (CH_2), 44.5 (CH_2), 49.8 (CH), 57.0 (CH), 72.4 (CH_2), 111.9-128.7, (Ph_{CH}), 131.8 ($Ph_{Cquaternary}$), 134.6 ($Ph_{Cquaternary}$), 136.8 ($Ph_{Cquaternary}$), 159.3 ($C_{quaternary}$), 166.8 (OC=O), 173.4 (NC=O), (1 x $Ph_{Cquaternary}$ not seen); m/z (+FAB) 420 (M^+ + H, 100%), 105 (57). (-FAB) 418 (M^+ - H, 100%), 258 (8), 227 (8), 135 (35), 92 (28).

3,4-Dehydroproline was synthesised according to a literature procedure from (2S,4R)-[N-(Cbz)]-4-hydroxyproline methyl ester (**63**), which was in turn prepared from (2S,4R)-4-hydroxyproline.[106]

(2S)-[N-(Benzyloxycarbonyl)]-3,4-dehydroproline (**67**)

To an ice-cooled solution of methyl ester (**66**) (13.6 g, 0.052 mol) in methanol (150 ml) was added 1M aqueous solution of lithium hydroxide (55 ml). The mixture was stirred at room temperature for 6h. After that time, most of the solvent (120 ml) was evaporated under reduced pressure. The mixture was than cooled to 0°C and acidified to pH1 with 5M aqueous hydrochloric acid. On acidification a yellow oil crashed out of solution. The mixture was then extracted into dichloromethane (5 x 100ml), dried (Na_2SO_4), filtered and evaporated *in vacuo* to give N-protected dehydroproline (**67**) (12.6 g, 98%) as an orange oil. υ_{max} (thin film) 3200-2851 (br), 1750-1660 (br) cm^{-1}; δ_H 42.8-4.36 (2H, m), 5.08-5.27 (3H, m), 5.76-5.90 (1H, m, vinyl H), 5.98-6.09 (1H, m, vinyl H), 6.60-6.90 (1H, s (br), OH), 7.30-7.40 (5H, m, Ph); m/z (C.I.) 248 (M^+ + H, 15%), 202 (28), 91 (100).

The overall yield from (2S,4R)-4-hydroxylproline to N-Cbz-dehydroproline was 86%. The ^1H NMR spectrum of (**67**) was checked against a commercially available sample of N-Cbz-dehydroproline.

N-Benzylhydroxylamine (**70**)[107]

N-Benzylhydroxylamine (**70**) was prepared according to literature procedure as colourless needles (ethyl acetate-petrol) m.p. 54-56°C (lit.,[107] 56-57°C); υ_{max} (nujol mull) 3260 and 3150 cm^{-1}; δ_H 3.99 (2H, s, $C\underline{H}_2Ph$), 5.60-5.75 (2H,

s(br), NH and OH), 7.33-7.35 (5H, m, Ph); m/z (E.I.) 123 (M^+, 80%) and 91 (100).

N-Benzyl-1-(benzyloxycarbonyl)-2,5-dihydro-N-hydroxy-1-pyrrole-carboxamide (72)

To an ice-cooled solution of [N-(Cbz)]-3,4-dehydroproline (**67**) (12.6 g, 0.05 mol) in dichloromethane (150 ml) was added N-benzylhydroxylamine (6.85 g, 0.055 mol), followed by addition of dicyclohexylcarbodiimide (11.8 g, 0.055 mol). After 5-10 min, a precipitate occurred. The mixture was stirred at room temperature for 20h. After that time, the dicyclohexylurea was filtered and washed with dichloromethane (5 x 30 ml). The filtrate was then washed with 1M aqueous hydrochloric acid (100 ml), followed by 1M aqueous potassium hydrogen carbonate solution (100 ml) and water (100 ml). The organic extract was dried (Na_2SO_4) and evaporated under reduced pressure to give an oil which was purified by chromatography using ethyl acetate-petrol (3:7 v/v) as eluant, to give pyrrolecarboxamide (**72**) (8.74 g, 49%) as a colourless oil (Found: M^+ + H, 353.1501. $C_{20}H_{21}N_2O_4$ requires 353.1501); υ_{max} ($CHCl_3$) 3150 (br), 2900, 1680-1640 (br) cm^{-1}; δ_H 4.17-4.37 (2H, m, $N\underline{CH_2}Ph$), 4.67 (1H, dd, *J* 15 and 5 Hz, vinyl H), 4.87-4.98 (1H, m, vinyl H), 5.00-5.12 (2H, m, $CO_2\underline{CH_2}Ph$), 5.65-5.69 (1H, m, 5-H), 5.80-5.85 (1H, m, 5-H), 5.95-6.00 (1H, m, 2-H), 7.29-7.34 (10H, m, Ph), 9.40-9.50 (1H, s (br), OH); δ_C 28.9 (CH), 33.8 (CH), 52.0 (CH_2), 53.7 (CH_2), 63.5 (CH), 67.6 (CH_2), 125.3-128.5 (Ph_{CH}), 135.9 ($Ph_{Cquaternary}$), 155.2 ($Ph_{Cquaternary}$), 168.2 ($C_{quaternary}$),(1 x C=O not seen); m/z (C.I.) 353 (M^+ + H, 4%) and 91 (100).

(4R)-Phenylseleno-2-[N-(benzyl)]-2H-pyrrolo[2,3-d]isoxazolidine-3-one (73)

To an ice-cooled solution of (72) (8.75 g, 0.025 mol) in dichloromethane (50 ml) was added phenylselenenyl chloride (4.55 g, 0.024 mol). As the reaction proceeded the solution changed from red to a yellow colour and the mixture was stirred at room temperature until all the starting material had disappeared (2h) as judged by TLC. Silver tetrafluoroborate (4.78 g, 0.024 mol) was then added to the ice-cooled solution and was further stirred in the dark for 1.5h at room temperature. The silver salts were removed by filtration through celite and the solids washed with dichloromethane (2 x 30 ml). The combined filtrate was poured into water (100 ml) and extracted into dichloromethane (3 x 100 ml). The extracts were dried (Na_2SO_4) and evaporated under reduced pressure to give an oil which was purified by chromatography using ethyl acetate-petrol (3:7 v/v) as eluant, to give the minor phenylselenenyl product (74) (1.94 g, 16%) followed by the isoxazolidinone (73) (8.1 g, 64%) as a yellow oil.

Isoxazolidinone (73): (Found: $M^+ + H$, 509.0980. $C_{26}H_{25}N_2O_4Se$ requires 509.0985); υ_{max} (thin film), 3050, 3020, 2970 (br), and 1690 cm^{-1}; δ_H (400 MHz, DMSO-d_6, 75°C) 3.65 (1H, dd, *J* 12.5 and 6 Hz, 5-H), 3.78 (1H, dd, *J* 12.5 and 2.5 Hz, 5-H), 3.91 (1H, dt, *J* 6 and 2 Hz, 4-H), 4.62 (1H, d, *J* 15.9 Hz, part of AB system), 4.69 (1H, d, *J* 15.9 Hz, part of AB system), 5.02 (1H, d(br), *J* 7 Hz, 2-H), 5.09 (1H, d, *J* 13.6 Hz, part of A'B' system), 5.10 (1H, dd, *J* 6.8 and 2.3 Hz, 3-H), 5.15 (1H, d, *J* 12.5 Hz, part of A'B' system), 7.25-7.39 (13H, m), 7.51-7.54 (2H, m); δ_C 41.6 (CH), 48.8 (CH_2), 51.0 (CH_2), 61.7 (CH), 67.6 (CH_2), 85.2 (CH), 127.6-129.5 (Ph_{CH}), 135.3 ($Ph_{Cquaternary}$), 135.5 ($Ph_{Cquaternary}$), 148.2 ($Ph_{Cquaternary}$), 160.8 (C=O), (1 x C=O not seen); m/z (C.I.) 509 ($M^+ + H$, 5%), 507 (3), 506 (2), 91 (60).

Compound (74): (Found $M^+ + H$, 465.1109. $C_{25}H_{25}N_2O_2Se$ requires

465.1076); υ_{max} (thin film) 3050, 3020, 2910, 2790 and 1680 cm^{-1}; δ_H (400 MHz, DMSO-d_6, 60°C) 2.80 (1H, dd, *J* 10.5 and 4.9 Hz, 5-H), 3.14 (1H, dd, *J* 10.5 and 5.9 Hz, 5-H), 3.72 (1H, ddd, *J* 6, 4.9 and 2.5 Hz, 4-H), 3.98 (1H,d, *J* 13.2 Hz, part of AB system), 4.05 (1H, d, *J* 13.2 Hz, part of AB system), 4.13 (1H, d, *J* 7 Hz, 2-H), 4.60 (1H, d, *J* 16 Hz, part of A'B' system), 4.72 (1H, d, *J* 16 Hz, part of A'B' system), 5.07 (1H, dd, *J* 7 and 2.3 Hz, 3-H), 7.28-7.35 (13H, m), 7.47-7.50 (2H, m); δ_C 44.7 (CH), 48.5 (CH_2), 54.8 (CH_2), 56.3 (CH_2), 66.2 (CH), 86.4 (CH), 128.0-133.8 (Ph_{CH}), 135.0 ($Ph_{Cquaternary}$), 138.2 ($Ph_{Cquaternary}$), 163.5 ($C_{quaternary}$), (1 x $C_{quaternary}$ not seen); m/z (C.I.) 465 (M^+ + H, 30%), 91 (100).

[N-(Benzyloxycarbonyl)-(*cis*)-hexahydro-2-[N-(benzyl)]-2H-pyrrolo[2,3-d]-isoxazolidine-3-one (**75**)

To a solution of (**73**) (8.1 g, 0.016 mol) in toluene (100 ml) in a sealed tube was added tributyltin hydride (11 ml, 0.047 mol), followed by a catalytic amount of azobisisobutyronitrile. The mixture was heated at 100°C for 5h. Removal of solvent followed by purification by chromatography, using ethyl acetate-petrol (1:1 v/v) as eluant, gave the deselenated cis-fused bicycle (**75**) (5.1 g, 91%) as a colourless oil; (Found: M^+ 352.1436. $C_{20}H_{20}N_2O_4$ requires 352.1423); υ_{max} (thin film) 3060, 3020, 2950 and 1690 cm^{-1}; δ_H 1.99-2.30 (2H, m, 5-CH_2), 3.16-3.3 (1H, m), 3.74-3.94 (1H, m (br)), 3.68 (2H, s, N$\underline{CH_2}$Ph), 4.85-5.06 (2H, m), 5.17-5.25 (2H, m, $CO_2\underline{CH_2}$Ph), 7.28-7.52 (10H, m, Ph x 2); δ_C 30.6 (CH_2), 44.8 (CH_2), 48.7 (CH_2), 62.6 (CH), 67.5 (CH_2), 81.0 (CH), 128.0-128.6 (Ph_{CH}), 134.7 ($Ph_{Cquaternary}$), 136.4 ($Ph_{Cquaternary}$), 164.5 (C=O), (1 x C=O not seen); m/z (E.I.) 352 (M^+, 100%) and 91 (63).

Using the above procedure, the minor adduct (**74**) (1.28 g, 2.7 mmol) was deselenylated to afford a compound (**75b**) (604 mg, 73%) as a yellow oil;

(Found M^+ + H, 309.1603. $C_{19}H_{21}N_2O_2$ requires 309.1603); υ_{max} ($CHCl_3$) 2920, 2840 and 1675 cm^{-1}; δ_H 1.90-1.99 (1H, m, 5-H), 2.02-2.12 (1H, m, 5-H), 2.64-2.89 (2H, m, 4-CH_2), 3.93 (1H, d, *J* 7.5 Hz, 2-H), 4.05 (1H, d, *J* 12 Hz, part of AB system), 4.15 (1H, d, *J* 13.5 Hz, part of AB system), 4.65 (1H, d, *J* 15 Hz, part of A'B' system), 4.75 (1H, d, *J* 16 Hz, part of A'B' system), 4.93 (1H, td, *J* 6.8 and 2.2 Hz, 3-H), 7.26-7.40 (10H, m, Ph x 2); δ_C 32.3 (CH_2), 48.3 (CH_2), 50.7 (CH_2), 55.6 (CH_2), 67.0 (CH), 81.0 (CH), 126.9-128.8 (Ph_{CH}), 135.1 ($Ph_{Cquaternary}$), 138.5 ($Ph_{Cquaternary}$), 166.1 (C=O); m/z (C.I.) 309 (M^+ + H, 100%), 216 (11), 91 (74). (+FAB) 309 (M^+ + H, 100%), 217 (8), 202 (5), 91 (98).

(cis)-Hexahydro-2-[N-(benzyl)]-2H-pyrrolo[2,3-d]isoxazolidine-3-one (**76**)

To a solution of isoxazolidine-3-one (**75**) (125 mg, 3.5 mmol) in methanol (5 ml) was added 20% palladium hydroxide over charcoal catalyst (20 mg). The mixture was then subjected to atmospheric hydrogenation for 34h. The catalyst was removed by filtration through celite and the solids washed with methanol (15 ml). The filtrate was evaporated under reduced pressure to give an oil which was purified by chromatography, using dichloromethane--methanol (9:1 v/v) as eluant, to give bicycle (**76**) (47 mg, 61%) as a colourless oil. (Found: M^+ 218.1052. $C_{12}H_{14}N_2O_2$ requires 218.1055); υ_{max} (thin film) 3300, 2920 and 1675 cm^{-1}; δ_H 1.90-2.07 (2H, m, 5-CH_2), 2.70-2.91 (2H, m, NH and 4-H), 3.15 (1H, ddd, *J* 9.8, 6.7 and 2.2 Hz, 4-H), 4.47 (1H, d, *J* 7 Hz, 2-H), 4.63 (1H, d, *J* 15.6 Hz, part of AB system), 4.74 (1H, d, *J* 15.4 Hz, part of AB system), 4.98-5.05 (1H, m, 3-H), 7.30-7.36 (5H, m, Ph); δ_C (Methanol-d_4) 28.9 / 29.0 (CH_2), 44.3 / 44.5 (CH_2), 57.4 / 57.5 (CH_2), 65.7 / 66.5 (CH), 81.9 / 82.4 (CH), 128.7-130.9 (Ph_{CH}), 139.5 ($Ph_{Cquaternary}$), 164.9 / 165.7 (C=O); m/z (70eV E.I.) 218 (M^+, 11%), 128 (8), 85 (100).

Attempt to N-debenzylate [N-(Benzyloxycarbonyl)]-(*cis*)-hexahydro--2-[N-(benzyl)]-2H-pyrrolo[2,3-d]isoxazolidine-3-one (75) with Iodotrimethylsilane

Iodotrimethylsilane was generated according to literature procedure[111] from trimethylsilylchloride (0.23 ml, 1.8 mmol) and anhydrous sodium iodide (4.10 mg, 2.7 mmol) in acetonitrile (5 ml). To this mixture was added bicycle (**75**) (144mg, 0.4 mmol) in acetonitrile (1.5 ml). The mixture was stirred at room temperature for 1h and then heated at reflux for 45 min, by which time compound (**76**) was observed by TLC along with the disappearance of starting material. The mixture was then further heated at reflux for 72h. On returning the solvent was removed *in vacuo* and the mixture suspended in dichloromethane and the solids were removed by filtration and the filtrate was evaporated under reduced pressure to afford an oil. The oil was purified by chromatography, using ethyl acetate-petrol (1:1 v/v) and dichloromethane--methanol (9:1 v/v) as eluant, to give isoxazolidine-3-one (**76**) (36 mg, 40%).

Other methods attempted to remove the N-benzyl residue from compound (**75**) and (**76**) included the Hard acid-Soft nucleophile approach (CF_3SO_3H and anisole)[104] and dissolving metal reduction (Na / NH_3)[112]. In both cases N-debenzylation was unsuccessful and in the former reaction the isoxazolidine-3-one (**76**) was isolated in 80% yield.

1-[N-(Benzyloxycarbonyl)-3-hydroxyl-N-(phenylmethyl)-2-pyrrolidine-carboxamide (77)

To a solution of isoxazolidine-3-one (**75**) (108 mg, 0.3 mmol) in methanol (5 ml) was added ammonium formate (81 mg, 1.3 mmol) and 10% palladium over charcoal (109 mg). The mixture was left to stir at room temperature.

After 3.5h, starting material (**75**) had disappeared and [N-(benzyl)]--pyrrolo[2,3-d]isoxazolidinone (**76**) was be seen by TLC. The mixture was then left to stir at room temperature for 20h. On returning, more ammonium formate (216 mg, 3.4 mmol) and catalyst (179 mg) were added and the reaction was left to stir at room temperature for another 24h. After that time, the catalyst was removed by filtration through celite and the solids washed with methanol (15 ml). The filtrate was evaporated under reduced pressure to give a colourless solid which was dissolved in 1M aqueous sodium bicarbonate (1 ml), cooled to 0°C and a solution of benzyl chloroformate (0.044 ml, 0.3 mmol) in dioxane (1 ml) was added. The mixture was stirred at 0°C for 0.5h and then at room temperature for 2h. After that time, the mixture was extracted with ether (2 x 10 ml), dried (Na_2SO_4) and concentrated *in vacuo* to afford an oil. The aqueous layer was cooled to 0°C and acidified to pH1 with 5M aqueous hydrochloric acid. The mixture was then extracted with dichloromethane (3 x 10 ml), dried (Na_2SO_4), filtered and evaporated under reduced pressure to give an oil. The two oils were combined and purified by chromatography using ethyl acetate-petrol as eluant, to give the 3-hydroxypyrrolidine (**77**) (30 mg, 28%) as a colourless solid, m.p. 131-133°C (ethyl acetate-petrol) (Found: C, 67.5; H, 6.17; N, 7.99. $C_{20}H_{22}N_2O_4$ requires C, 67.78; H, 6.25; N, 7.90%): υ_{max} (nujol mull) 3280, 1705, 1690 and 1645 cm^{-1}; δ_H 2.05-2.16 (2H, m, 4-CH_2), 2.28-2.45 (2H, m, 5-CH_2), 3.53-3.79 (2H, m, 2-H and NH or OH), 4.37-4.62 (4H, m, N$\underline{CH_2}$OPh, 3-H, NH or OH), 5.14 (2H, s, $CO_2C\underline{H_2}Ph$), 7.27-7.37 (10H, m, Ph x 2); m/z (C.I.) 355 (M^+ + H, 10%), 107 (3), 91 (100).

1-[N-(4-Bromobenzoyl)]-(*cis*)-Hexahydro-2-[N-(benzyl)]-2H-pyrrolo[2,3-d]-isoxazolidine-3-one (78)

To an ice-cooled solution of isoxazolidine-3-one (**76**) (256 mg, 1.1 mmol) in THF (8 ml) was added 4-bromobenzoyl chloride (286 mg, 1.3 mmol) and 4-dimethylaminopyridine (162 mg, 1.3 mmol). The mixture was stirred at 0°C for 15 min and then at room temperature for 20h. After that time, the solvent was evaporated under reduced pressure and the crude solid was purified by chromatography using ethyl acetate- petrol as eluant, to afford the N-(4-bromobenzoyl) derivative (**78**) as a solid. Recrystallisation from ethyl acetate afforded (**78**) (153 mg, 38%) as a colourless needles, m.p. 143-145°C (Found: C, 56.8; H, 4.23; N, 6.98. $C_{19}H_{17}BrN_2O_3$ requires C, 56.85; H, 4.27; N, 6.98%); υ_{max} (nujol mull) 1680 and 1620 cm^{-1}; δ_H 2.0-2.10 (2H, m, (br), 4-CH_2), 4.62-4.82 (4H, m, 5-CH_2 and $C\underline{H}_2Ph$), 5.02-5.10 (2H, m), 7.36 (5H, s, Ph), 7.58 (4H, d, *J* 6 Hz, CO$\underline{Ph}$-Br); m/z (70eV E.I.) 402 (M^+, 100%), 400 (10), 185 (98), 183 (100), 91 (95). (E.I.) 402 / 400 (M^+, 30%), 280 (95), 278 (100).

1-[N-(Benzyloxycarbonyl)]-(4R)-hydroxyl-N-(phenylmethoxy)-(2S)--pyrrolidinecarboxamide (80)

(2S,4R)-[N-(Cbz)]-4-hydroxyproline (**62**) (1.5 g, 5.7 mmol) was coupled with O-benzylhydroxylamine (707 mg, 5.7 mmol) in the presence of dicyclohexylcarbodiimide (1.34 g, 6.4 mmol) in dichloromethane (30 ml) under standard peptide coupling procedure.[105] The pyrrolidinecarboxamide derivative (**80**) (1.1 g, 52%) was isolated as a colourless solid, m.p. 130-131°C (EtOAc-Petrol) (Found: C, 64.88; H, 6.02; N, 7.58. $C_{20}H_{22}N_2O_5$ requires C, 64.85; H, 5.99; N, 7.56%); υ_{max} (nujol mull) 3470, 3260, 1690 and 1675 cm^{-1}; δ_H 2.13-2.25 (1H, m, 3-H), 2.50-2.60 (1H, m, 3-H), 3.49-3.57 (2H, m, 5-CH_2),

4.26-4.34 (1H, m), 4.50-4.58 (1H, m), 4.86-4.93 (2H, m, NCH_2Ph), 5.05 (1H, d, *J* 12.5 Hz, part of AB system), 5.10 (1H, d, *J* 12.6 Hz, part of AB system), 7.32-740 (10H, m, Ph x 2), 9.57 (1H, s, NH / OH), (1 x NH / OH not seen); m/z (+FAB) 371 (M^+ + H, 40%), 91 (100); (-FAB) 369 (M^+ - H, 100%), 352 (20), 272 (5), 256 (5), 93 (6).

1-[N-(Benzyloxycarbonyl)]-(4R)-Hydroxyl-N-(phenylmethoxy)-(2S)--pyrrolidinecarboxamide (**81**)

To an ice-cooled solution of pyrrolidinecarboxamide (**80**) (500 mg, 1.3 mmol) in pyridine (3 ml) was added methanesulphonyl chloride (0.1 ml, 1.3 mmol). The mixture was stirred at 0°C for 4h and then left in a freezer (-23°C) for 20h. On returning, the mixture was diluted with dichloromethane (30 ml) and washed with 1M aqueous hydrochloric acid (20 ml) and water (2 x 20 ml). The dichloromethane extract was dried (Na_2SO_4) and evaporated under reduced pressure to give a solid which was recrystallised from ethyl acetate to afford the mesylated product (**81**) (408 mg, 65%) as a colourless solid, m.p. 130-132°C (Found: C, 56.2; H, 5.33; N, 6.2. $C_{21}H_{24}N_2O_7S$ requires C, 56.25; H, 5.40; N, 6.24%); υ_{max} (nujol mull) 3400, 1705 and 1670 cm^{-1}; δ_H 2.27-2.38 (1H, m, 3-H), 2.76-2.87 (1H, m, 3-H), 2.98 (3H, s, SO_2Me), 3.60-3.68 (1H, m, 5-H), 3.92 (1H, d, *J* 12 Hz, 5-H), 4.34 (1H, t, *J* 7.5 Hz, 2-H), 4.86 (1H, d, *J* 11.5 Hz, part of AB system), 4.93 (1H, d, *J* 11.2 Hz, part of AB system), 5.06 (1H, d, *J* 12.5 Hz, part of A′B′ system), 5.14 (1H, d, *J* 12.3 Hz, part of A′B′ system), 5.24-5.33 (1H, m, 4-H), 7.31-7.42 (10H, m, Ph x 2), 9.46 (1H, s, NH); m/z (+FAB) 449 (M^+ + H, 9%), 207 (4), 91 (51).

(4R)-Methanesulphonyloxy-N-(hydroxy)-(2S)-pyrrolidinecarboxamide (**82**)

To a solution of pyrrolidinecarboxamide (**81**) (12.26 g, 0.027 mmol) in methanol (700 ml) was added 10% palladium over charcoal catalyst (2.48 g). The mixture was then subjected to atmospheric hydrogenation for 3h and, after which time, the solution was filtered through celite and the solids were washed with methanol (200 ml). The filtrate was evaporated under reduced pressure to give a brown oil/solid which was then suspended in dichloromethane (50 ml) and filtered. The filtered solid was found to be the doubly deprotected material (**82**) (5.2 g, 86%) m.p. 118-119°C (MeOH) (Found: C, 32.13; H, 5.40; N, 12.1. $C_6H_{12}N_2O_5S$ requires C, 32.15; H, 5.39; H, 12.49%); υ_{max} (nujol mull) 3260, 3200 and 1650 cm^{-1}; δ_H (DMSO-d_6) 1.95-2.05 (1H, m, 3-H), 2.12-2.21 (1H, m, 3-H), 2.94-3.02 (1H, m, 5-H), 3.10-3.21 (4H, m, SO_2Me and 5-H), 3.61 (1H, t, *J* 8 Hz, 2-H), 5.18-5.21 (1H, m, 4-H), (2 x NH and 1 x OH not seen); m/z (E.I.) 224 (M^+, 100%), 164 (42).

NOTE: The filtrate was evaporated under reduced pressure and ^1H NMR showed that it was a mixture.

An attempt to cyclise (4R)-Methanesulphonyloxy-N-(hydroxy)-(2S)-
-pyrrolidinecarboxamide (**82**) with Triethylamine

To an ice-cooled solution of hydroxamic acid (**82**) (238 mg, 1.0 mmol) in methanol (4 ml) was added dropwise triethylamine (2 ml). The mixture was then left in a freezer (-23°C) for 48h. After that time, TLC (reverse phase) showed that only starting material was present. The mixture was then left to stir at room temperature for 2 days, but again, only starting material was observed and isolated, as proven by ^1H NMR.

1-[N-(*tert*-Butyloxycarbonyl)]-(4R)-Methanesulphonyloxy-N-(hydroxy)-(2S)--pyrrolidinecarboxamide (**83**)

To an ice-cooled suspension of hydroxamic acid (**82**) (101 mg, 0.45 mmol) in THF/water (2:1) (5 ml) was added di-*tert*-butyldicarbonate (109 mg, 0.5 mmol). The mixture was stirred at room temperature for 20h. After that time, the solvent was removed *in vacuo* to give a solid which was suspended in dichloromethane (3 ml) and filtered to give the N-BOC material (**83**) (56 mg) m.p. 161-166°C (EtOH) (Found: C, 40.9; H, 6.29; N, 8.65. $C_{11}H_{20}N_2O_7S$ requires C, 40.74; H, 6.22; N, 8.63%); υ_{max} (nujol mull) 3360, 3200 and 1655 cm^{-1}; δ_H (Methanol-d_4) 1.45 (9H, s, $COMe_3$), 2.21-2.34 (1H, m, 3-H), 2.46-2.58 (1H, m, 3-H), 3.12 (3H, s, SO_2Me), 3.66-3.86 (2H, m, 5-CH_2), 4.13-4.27 (1H, m, 2-H), 5.29-5.34 (1H, m, 4-H), (1 x NH and 1 x OH not seen); m/z (+FAB) 325 (M^+ + H, 38%), 308 (14), 269 (100), 225 (61), 165 (23).

1-[N-(2,2,2-Trichloroethyloxycarbonyl)]-(2S,4R)-Hydroxylproline (**85**)

(2S,4R)-4-Hydroxyproline (1.73 g, 0.013 mol) was selectively N-protected with 2,2,2,-trichloroethyl chloroformate in the presence of 1M aqueous sodium bicarbonate solution (26 ml) at 0°C for 1.5h and then at room temperature for another 1.5h, as described in the synthesis of (4R)--[N-(FMOC)amino]-3-isoxazolidinone (**14**). [N-(TROC)]-4-Hydroxy-proline (**85**) was isolated as an oil (3.89 g, 97%); υ_{max} (nujol mull) 3300 (br), 1725 and 1670 cm^{-1}; δ_H (DMSO-d_6) 1.90-2.23 (1H, m, 3-H), 2.12-2.28 (1H, m, 3-H), 3.35-3.58 (2H, m, 5-CH_2), 4.20-4.36 (2H, m, 2-H and 4-H), 4.77 (1H, d, *J* 12.3 Hz, part of AB system), 4.88 (1H, d, *J* 12.8 Hz, part of AB system), 5.17-5.30 (1H, s (br), OH), (1 x OH not seen); m/z (C.I.) 310 (M^+ + H, 20%), 308 (M^+ + H, 50%), 306 (M^+ + H, 56%), 276 (8), 274 (40), 277 (68), 272 (68).

1-[N-(2,2,2-Trichloroethyloxycarbonyl)]-(4R)-Hydroxyl-N-(phenylmethoxy)--(2S)-pyrrolidinecarboxamide (86)

[N-(TROC)]-(2S,4R)-4-Hydroxyproline (**85**) (281 mg, 0.9 mmol) was coupled with O-benzylhydroxylamine (124 mg, 1 mmol) as described for the conversion of [N-(Cbz)]-hydroxyproline (**62**) to the O-benzylhydroxamic acid (**80**). Pyrrolidine (**86**) (203 mg, 55%) was isolated as a colourless solid, m.p. 161-162°C (ethyl acetate-petrol) (Found: C, 43.6; H, 4.05; N, 6.65. $C_{15}H_{17}Cl_3N_2O_5$ requires C, 43.76; H, 4.16; N, 6.80%); υ_{max} (nujol mull) 3370, 3250, 1700 and 1670 cm^{-1}; δ_H (DMSO-d_6) 2.05-2.15 (2H, m, 3-CH_2), 3.42-3.60 (2H, m, 5-CH_2), 4.18-4.36 (2H, m), 4.63-4.95 (4H, m, O$\underline{CH_2}$Ph and $\underline{CH_2}CCl_3$), 5.10-5.21 (1H, s (br), OH), 7.37-7.39 (5H, s (br), Ph), 11.34-11.36 (1H, m, NH); m/z (C.I.) 415 (M^+ + H, 1.7%), 413 (M^+ + H, 5%), 411 (M^+ + H, 6%), 379 (0.9), 377 (1.4), 262 (14), 107 (46), 91 (100).

1-[N-(2,2,2-Trichloroethyloxycarbonyl)]-(4R)-Methanesulphonyloxy-N--(phenylmethoxy)-(2S)-pyrrolidinecarboxamide (87)

O-Benzylhydroxamic acid (**86**) (1.35 g, 3 mmol) was O-mesylated with methanesulphonyl chloride (0.28 ml, 36 mmol) in pyridine (3.5 ml) at -23°C, for 20h. Pyrrolidinecarboxamide (**87**) (1.4g, 96%) was isolated as a colourless oil, following the procedures employed for the conversion of the [N-(Cbz)] derivative (**80**) to (**81**); υ_{max} ($CHCl_3$) 3350 (br), 2920 (br) and 1710 cm^{-1}; δ_H 2.36-2.48 (1H, m, 3-H), 2.79-2.89 (1H, m, 3-H). 3.06 (3H, s, SO_2Me), 3.74 (1H, dd, *J* 12.7 and 4.5 Hz, 5-H), 4.00-4.57 (1H, m, 5-H), 4.34 (1H, t, *J* 7.5 Hz, 2-H), 4.62 (1H, d, *J* 11.9 Hz, part of AB system), 4.80 (1H, d, *J* 12.1 Hz, part of AB system), 4.90-4.99 (2H, m), 5.31-5.37 (1H, m), 7.39 (5H, s, Ph), 10.5 (1H, s, NH); m/z (C.I.) 491/489 (M^+ + H, 0.4%), 395 (13), 393 (14), 359 (7), 91 (100).

1-[N-(2,2,2-Trichloroethyloxycarbonyl)]-(4R)-Methanesulphonyloxy--N-(hydroxy)-(2S)-pyrrolidinecarboxamide (88)

To a solution of pyrrolidine (87) (217 mg, 0.44 mmol) in methanol (5 ml) was added 10% palladium over charcoal catalyst (47 mg). The mixture was then subjected to atmospheric hydrogenation for 20h. After that time, the catalyst was removed by filtration through celite and the solids washed with methanol (20 ml). The filtrate was evaporated under reduced pressure to give the hydroxamic acid (88) (122 mg, 69%) as an oil which later solidified and was recrystallised from ethyl acetate as a colourless solid, m.p. 168.6-169.1°C (Found: C, 27.6; H, 3.38; N, 6.84. $C_9H_{13}Cl_3N_2O_7S$ requires C, 27.05; H, 3.28; N, 7.00%); υ_{max} (nujol mull) 3340, 3250 and 1675 cm^{-1}; δ_H (DMSO-d_6) 2.14.2.28 (1H, m, 3-H), 2.38-2.46 (1H, m, 3-H), 3.25 (3H, s, SO_2Me), 3.65-3.85 (2H, m), 4.13-4.20 (1H, m), 4.77-4.93 (2H, m, $C\underline{H}_2CCl_3$), 5.27-5.38 (1H, m), 9.19 (1H, s, NH), 10.14 (1H, s, OH); m/z (+FAB) 399 (M^+ + H, 91%), 338 (20), 244 (36).

NOTE: Subsequent runs afforded (88) in the range of 69-96% yield.

Octahydro-7-[(methanesulphony)oxy]-pyrrolo[1,2-d][1,2,5]oxadiazine (88b) from cyclisation of 1-[N-(2,2,2-trichloroethyloxycarbonyl)]--(4R)-methanesulphonyloxyN-(hydroxy)-(2S)-pyrrolidinecarboxamide (88) with Triethylamine

To a solution of hydroxamic acid (88) (85 mg, 0.21 mmol) in methanol (1.5 ml) was added triethylamine (0.03 ml, 0.23 mmol. TLC of the mixture after 1.5h at room temperature showed mostly starting material. The mixture was then heated at 60°C for 2h. After that time, the solvent was removed under reduced pressure to give an oil. The oil was dissolved in dichloromethane (10

ml) and washed with saturated aqueous ammonium chloride solution (2 ml). The dichloromethane extract was dried (Na_2SO_4) and concentrated *in vacuo* to give an oil which was purified by chromatography, using dichloromethane--methanol (9:1 v/v) as eluant, to give oxadiazine (**88b**) (14 mg, 27%) as an oil; υ_{max} ($CHCl_3$) 3260 (br), 2910, 1785 and 1725 cm^{-1}; δ_H (DMSO-d_6) 2.01 (1H, ddd, *J* 13.9, 11.4 and 5.5 Hz, 3-H), 2.47 (1H, dd, *J* 13.9 and 6.6 Hz, 3-H), 3.06 (3H, s, SO_2Me), 3.41 (1H, d, *J* 13.2 Hz, 5-H), 3.90 (1H, dd, *J* 13.1 and 5.5 Hz, 5-H), 4.34 (1H, dd, *J* 11 and 6.6 Hz, 2-H), 5.39 (1H, t, *J* 5.1 Hz, 4-H), (1 x NH not seen); δ_C (DMSO-d_6) 34.0 (CH_2), 37.9 (CH_3), 52.3 (CH_2), 59.1 (CH), 82.5 (CH), 157.7 ($C_{quaternary}$), (1 x C=O not seen); m/z (+FAB) 251 (M^+ + H, 1.4%), 149 (100); (-FAB) 249 (M^+ - H, 26%), 95 (100).

An accurate molecular ion was not found for oxadiazine (**88b**).

NOTE: Heating a solution of (**88**) (86 mg, 0.21 mmol) in dimethylformamide (1 ml) in the presence of sodium hydride (60% dispersed in oil, 10 mg, 0.25 mmol) for 2h at 50°C also afforded (**88b**) (19 mg, 36%) as an oil from chromatography.

1-[N-(2,2,2-Trichloroethyloxycarbonyl)]-(4R)-Hydroxyl-N-(hydroxy)--(2S)-pyrrolidinecarboxamide (**89**)

To a solution of pyrrolidine (**86**) (238 mg, 0.57 mmol) in methanol (5 ml) was added 10% palladium over charcoal catalyst (49 mg). The mixture was then subjected to atmospheric hydrogenation for 2h. After that time, the catalyst was removed by filtration through celite and the solids washed with methanol (20 ml). The filtrate was evaporated under reduced pressure to give hydroxamic acid (**89**) as an oil (178 mg, 97%); υ_{max} ($CHCl_3$) 3400 (br), 2930, 1700 (br) cm^{-1}; δ_H (Methanol-d_4) 2.05-2.28 (2H, m (br), 3-CH_2), 3.56-3.68 (2H, m (br), 5-CH_2), 4.38-4.48 (2H, m, 2-H and 4-H), 4.69-4.85 (2H, m,

$C\underline{H}_2CCl_3$), (1 x NH and 1 x OH not seen); m/z (C.I.) 322/320 (M^+, 0.8%), 290 (0.7), 288 (1), 287 (0.8), 286 (0.7).

(2S,4R)-[N-(1-oxo-2,2-Dimethyloxopropyl)]hydroxyproline (**91**)

To an ice-cooled solution of (2S,4R)-4-Hydroxyproline (580 mg, 4.4 mmol) in 1M aqueous sodium bicarbonate (8.8 ml, 8.8 mmol) was added a solution of trimethylacetyl chloride (0.6 ml, 4.8 mmol) in dioxane (5 ml) over a period of 2h. The mixture was further stirred at 0°C for 4h and then at room temperature for 20h. After that time, the mixture was extracted with ether (2 x 10 ml) and the aqueous layer cooled to 0°C and acidified with 5M aqueous hydrochloride acid to pH1. The product was extracted into ethyl acetate (6 x 20 ml), dried (Na_2SO_4) and concentrated *in vacuo* to give the [N-(1-oxo-2,2--dimethylpropyl)]hydroxyproline (**91**) as a colourless solid. Recrystallisation from ethanol-ether afforded (**91**) (548 mg, 54%) m.p. 169-170°C (Found: C, 56.1; H, 8.1; N, 6.57. $C_{10}H_{17}NO_4$ requires C, 55.8; H, 7.96; N, 6.50%); υ_{max} (nujol mull) 3250, 1735 cm^{-1}; δ_H (Methanol-d_4) 1.25 (9H, s, $COCMe_3$), 1.95 (1H, ddd, *J* 13.5, 9.0 and 4.5 Hz, 3-H), 2.15-2.26 (1H, m, 3-H), 3.73-3.89 (2H, m, 5-CH_2), 4.46-4.55 (2H, m, 2-H and 4-H), (2 x OH not seen); m/z (C.I.) 216 (M^+ + H, 100%), 200 (15), 154 (20), 86 (24).

Large scale preparation of 1-[N-(1-oxo-2,2-dimethylpropyl)]-(4R)-Hydroxyl--N-(phenylmethoxy)(2S)-pyrrolidinecarboxamide (**92**)

To an ice-cooled solution of (**91**) (40.8 g, 0.19 mmol) in dichloromethane (1 l) and dimethylformamide (15 ml) was added portionwise 1-hydroxybenzo--triazole hydrate (33.4 g, 0.24 mol) followed by dicyclohexylcarbodiimide (47.0 g, 0.23 mol). The suspension was stirred at 0°C for 1.25h, followed by dropwise addition of a solution of O-benzylhydroxylamine (28.6 g, 0.23 mol)

in dichloromethane (100ml). A further 400 ml of dichloromethane was added to the mixture and the mixture was stirred at 0°C for another 1h and then at room temperature for 20h. After that time, the dicyclohexylurea was removed by filtration and the solids washed with dichloromethane (800 ml). The filtrate was then washed with 2M aqueous hydrochloric acid (500 ml), 1M aqueous sodium bicarbonate (600 ml) and brine (600 ml). The dichloromethane extract was dried (Na_2SO_4), filtered and evaporated under reduced pressure to give, after recrystallisation from ethyl acetate, N-(phenylmethoxy)pyrrolidinecarboxamide (**92**) (40.89 g, 67%) as a colourless solid, m.p. 176-177°C (Found: C, 63.60; H, 7.61; N, 8.72. $C_{17}H_{24}N_2O_4$ requires C, 63.73; H, 7.55; N, 8.74%); υ_{max} (nujol mull) 3240, 3250 (br), 3140, 1670 and 1620 cm^{-1}; δ_H 1.21 (9H, s, $COCMe_3$), 2.04-2.22 (2H, m, 3-CH_2), 3.70 (1H, dd, *J* 11 and 4 Hz, 5-H), 3.90 (1H, d, *J* 11 Hz, 5-H), 4.44-4.57 (2H, m, 2-H and OH), 4.90 (2H, s, $C\underline{H_2}Ph$), 5.57 (1H, d, *J* 10.6 Hz, 4-H), 7.34-7.41 (5H, m, Ph), 10.02 (1H, s, NH); m/z (C.I.) 321 (M^+ + H, 0.5%), 198 (48), 170 (60), 152 (31), 91 (100).

1-[N-(1-oxo-2,2-dimethylpropyl)]-(4R)-Methanesulphonyloxy-N--(phenylmethoxy)-(2S)-pyrrolidinecarboxamide (**93**)

4-Hydroxyl-2-pyrrolidinecarboxamide (**92**) (56.3 g, 0.17 mmol) was O-mesylated with methanesulphonyl chloride (0.015 ml, 0.19 mmol) using procedure described earlier for the synthesis of (**81**). Mesylate (**93**) (46 mg, 67%) was isolated as a colourless oil (Found: M^+ + H, 399.1590. $C_{18}H_{27}N_2O_6S$ requires 399.1590); υ_{max} (thin film) 3420 (br), 3200, 2960, 2220, 1680 and 1610 cm^{-1}; δ_H 1.22 (9H, s, $COCMe_3$), 2.17-2.29 (1H, m, 3-H), 2.76-2.83 (1H, m, 3-H), 3.04 (3H, s, SO_2Me), 3.69-3.77 (1H, m, 5-H), 4.24 (1H, d, *J* 12.5 Hz, 5-H), 4.61-4.68 (1H, m), 4.86-4.94 (2H, m, $C\underline{H_2}Ph$), 5.34-5.40 (1H, m), 7.35-7.43 (5H, m, Ph), 10.45 (1H, s, NH); δ_C 27.2 ($C\underline{Me_3}$),

32.2 (CH_2), 38.3 (SO_2Me), 53.8 (CH_2), 56.9 (CH), 73.0 (CH_2), 79.3 (CH), 128.5-129.0 (Ph_{CH}), 135.1 ($Ph_{Cquaternary}$), 168.7 (C=O), 178.2 (C=O), (1 x $\underline{C}Me_3$ not seen); m/z (+FAB) 399 (M^+ + H, 13%), 276 (100), 91 (41); (-FAB) 397 (M^+ - H, 2.4%), 95 (100).

Synthesis of 1-[N-(1-oxo-2,2-dimethylpropyl)]-(4R)-Methanesulphonyloxy--N-(phenylmethoxy)-(2S)-pyrrolidinecarboxamide (93) from 1-[N-(TROC)]--(4R)-methanesulphonyloxy-N-(phenylmethoxy)-(2S)-pyrrolidinecarboxamide (87)

To a solution of [N-(TROC)]-pyrrolidinecarboxamide derivative (**87**) (221 mg, 0.45 mmol) in acetic acid (3 ml) was added zinc powder (218 mg) and the mixture was stirred at room temperature for 20h. After that time the solids were removed by filtration through celite and the solids washed with methanol (15 ml). The filtrate was evaporated under reduced pressure to give an oil. The oil was dissolved in dichloromethane (15 ml) and washed with 1M aqueous sodium bicarbonate (2 ml), dried (Na_2SO_4) and concentrated *in vacuo* to give an oil. The oil was redissolved in dichloromethane (4 ml), cooled to 0°C and trimethylacetyl chloride (0.06 ml, 0.45 mmol) and pyridine (0.036 ml, 0.45 mmol) was added. The mixture was stirred at 0°C for 3.5h. After that time, another 7 ml of dichloromethane was added to the mixture and the mixture was washed with 1M aqueous hydrochloric acid (2 x 4 ml). The organic layer was separated, dried (Na_2SO_4) and evaporated under reduced pressure to give an oil (340 mg). Purification by chromatography using ethyl acetate-petrol as eluant, gave the [N-(1-oxo-2,2-dimethylpropyl)] adduct (**93**) (106 mg, 59%) as a colourless oil. ^1H NMR was identical to that of (**93**) derived from (**92**).

1-[N-(1-oxo-2,2-Dimethylpropyl)]-(4R)-methanesulphonyloxy-N-(hydroxy)--(2S)-pyrrolidinecarboxamide (94)

O-Benzylhydroxamic acid (93) (53 mg, 1.3 mmol) was O-debenzylated under an atmosphere of hydrogen in the presence of palladium over charcoal catalyst (122 mg), using the same procedure described earlier for the debenzylation of [N-(TROC)]-(4R)-methanesulphonyloxy-N-(phenylmethoxy)-(2S)--pyrrolidinecarboxamide (87). Hydroxamic acid (94) (41 mg, 100%) was isolated as an oil, which later solidified and was recrystallised from ethanol to give (94) as a colourless solid, m.p. 156.5-157.5°C (Found: C, 42.7; H, 6.57; N, 9.08. $C_{11}H_{20}N_2O_6S$ requires C, 42.85; H, 6.54; N, 9.08%); υ_{max} ($CHCl_3$) 3320 (br), 2930 (br), 1673 and 1610 cm^{-1}; δ_H (DMSO-d_6) 1.15 (9H, s, $COCMe_3$), 1.80-2.10 (1H, m, 3-CH), 2.23-2.34 (1H, m, 3-CH), 3.25 (3H, s, SO_2Me), 3.78-3.85 (1H, m, 5-H), 4.04-4.11 (1H, m, 5-H), 4.22-4.32 (1H, m), 5.34-5.40 (1H, m), 10.14 (1H, s, NH / OH), (1 x NH / OH not seen); m/z (+FAB) 309 ($M^+ + H$, 26%), 276 (100), 248 (29).

1-[N-(1-oxo-2,2-Dimethylpropyl)]-3-(hydroxyimino)-2-oxa-5-azabicyclo-[2.2.1]heptane (95)

To an ice-cooled solution of pyrrolidine (94) (2.92 g, 9.5 mmol) in dimethylformamide (13 ml) was added sodium hydride (60% dispersion in oil, 424 mg, 10 mmol). After stirring at 0°C for 15 min, a thick suspension formed and the mixture was then warmed to 60°C and then allowed to stir at room temperature for 20h. After that time, the dimethylformamide was evaporated under reduced pressure and the mixture was dissolved in dichloromethane (30 ml) and washed with saturated ammonium chloride solution (20 ml). The aqueous layer was further extracted with dichloromethane (20 ml) and the combined organic extracts were dried (Na_2SO_4) and concentrated *in vacuo* to

give an oil. Purification by chromatography using ethyl acetate-petrol (4:6 v/v) to (7:3 v/v) as eluant, gave azabicycle (**95**) (670 mg, 33%) as colourless needles, m.p. 208-209°C (ethanol-ether), $[\alpha]^{22.5}_D$ -48.23° (c 0.51 in MeOH) (Found: C, 56.9; H, 7.77; N, 13.2. $C_{10}H_{16}N_2O_3$ requires C, 56.59; H, 7.60; N, 13.19%); υ_{max} (nujol mull) 3360 (br), 1680 cm^{-1}; δ_H (DMSO-d_6) 1.17 (9H, s, COC<u>Me</u>$_3$), 1.95-2.10 (2H, m, 3-CH_2), 3.40-3.65 (2H, m, 5-CH_2), 5.10-5.20 (2H, m, 2-H and 4-H), 9.62 (1H, s, NH); δ_C (DMSO-d_6) 27.4 (C<u>Me</u>$_3$), 52.9 (CH_2), 57.6 (CH), 79.1 (CH), 155.7 (<u>CO</u>CMe$_3$), 174.8 (NC=O), (1 x <u>C</u>Me$_3$ and 1 x CH_2 not seem); m/z (70eV E.I.). 212 (M^+, 4.6%), 86 (6); (C.I.) 213 (M^+ + H, 13%), 197 (100), 170 (76), 85 (30).

NOTE: Azabicycle (**95**) was similarly obtained by heating hydroxamic acid (**94**) (61 mg, 0.19 mmol) with triethylamine (0.05 ml, 0.35 mmol) in THF (4 ml) at 80-100°C for 6 days. Purification by chromatography afforded (**95**) (3.4 mg, 7%) followed by starting material (**94**) (14 mg, 22%). Azabicycle (**95**) obtained from this method was verified by comparison of ^1H NMR spectra will that obtained from the above reaction.

<u>1-[N-Benzyloxycarbonyl)]-2-(hydroxyimino)-2-oxa-5-azabicyclo[2.2.1]-heptane (99)</u>

Azabicycle (**95**) (53 mg, 0.25 mmol) was dissolved in trifluoroacetic acid and water (4:1) (2 ml) and heated at 70-90°C for 1.5h. After this time, the solvent was evaporated under reduced pressure to give an oil which was then dissolved in 1M aqueous sodium bicarbonate solution (0.5 ml), cooled to 0°C and a solution of benzyl chloroformate (0.04 ml, 0.28 mmol) in dioxane (1 ml) was added dropwise, over a period of 10 min. The mixture was stirred at 0°C for 4h and at room temperature for 20h. After that time, the mixture was washed with ether (2 x 5 ml) and the aqueous layer was cooled to 0°C and

acidified to pH1 with 5M aqueous hydrochloric acid. The aqueous mixture was then extracted with ethyl acetate (5 x 5 ml) and separated. The organic extracts were dried (Na_2SO_4) and concentrated *in vacuo* to give an oil. Purification by chromatography, using ethyl acetate-petrol as eluant, afforded the N-benzyloxycarbonyl derivative of azabicycle (**99**) (24 mg, 37%) as an oil (Found: M^+ + H, 263.1032. $C_{13}H_{15}N_2O_4$ requires 263.1032); υ_{max} ($CHCl_3$) 3350 (br), 2920, 1780 and 1670 cm^{-1}; δ_H (Methanol-d_4) 1.94-2.08 (1H, m, 3-H), 2.26-2.40 (1H, m, 3-H), 3.27-3.36 (1H, m, 5-H), 3.52-3.60 (1H, m, 5-H), 4.22-4.32 (2H, m, 2-H and 4-H), 4.97-5.14 (2H, m, $\underline{CH_2}$Ph), 7.23-7.28 (5H, m, Ph), (1 x OH not seen); m/z (C.I) 263 (M^+ + H, 5%), 202 (15), 108 (14), 91 (100).

1-[N-(1-oxo-2,2-dimethylpropyl)]-(4R)-hydroxyl-N-(hydroxy)-(2S)--pyrrolidinecarboxamide (**100**)

O-Benzylhydroxamic acid (**92**) (5.26 g, 16 mmol) in methanol (100 ml) was O-debenzylated under atmospheric hydrogenation conditions in the presence of 10% palladium over charcoal catalyst (1.03 g) for 20h. After that time, the catalyst was removed by filtration through celite and the solids washed with methanol (50 ml). The filtrate was evaporated under reduced pressure to give hydroxamic acid (**100**) (2.79 g, 74%) as colourless needles, m.p. 188-190°C (ethanol-ether) (Found: C, 52.5; H, 8.02; N, 12.0. $C_{10}H_{18}N_2O_4$ requires C, 52.16; H, 7.88; N, 12.16%) υ_{max} (nujol mull) 3460, 3250 (br), 1655 and 1625 cm^{-1}; δ_H (DMSO-d_6) 1.14 (9H, s, $C\underline{Me_3}$), 1.68-1.81 (1H, m, 3-CH), 1.82-194 (1H, m, 3-CH), 3.54-3.68 (2H, m, 5-CH_2), 4.23 (1H, t, *J* 8 Hz,2-H), 4.28-4.37 (1H, m 4-H), 5.02 (1H, d, *J*, 3 Hz, 4-OH), 8.75 (1H, s, NH), 11.24 (1H, s, NOH); m/z (+FAB) 231 (M^+ + H, 72%), 198 (100), 170 (33), 86 (25). (-FAB) 229 (M^+ - H, 100%).

1-{[1-(t-Butylcarbonyl)-pyrrolidin-2-yl]carbonylamino-oxy}-1-
-(ethoxycarbonyamino)carbamic acid, ethyl ester (**101**)

To a cooled suspension of hydroxamic acid (**100**) (123 mg, 0.53 mmol) in THF (5 ml) was added triphenylphosphine (284 mg, 1 mmol) followed by dropwise addition of diethylazodicarboxylate (0.17 ml, 1 mmol) which gave a yellow solution. The mixture was stirred at 0°C for 1h. After that time, the solvent was removed *in vacuo* and the mixture was purified by chromatography using ethyl acetate-petrol as eluant, to afford triphenyl-
-phosphine oxide, followed by ester (**101**) (125 mg, 58%) as a colourless oil; υ_{max} (thin film) 3440 (br), 2980, 1790, 1740 and 1620 cm^{-1}; δ_H 1.24 (9H, s, $COCMe_3$), 1.40 (3H, t, *J* 7 Hz, $C\underline{H}_3CH_2$), 1.46 (3H, t, *J* 7 Hz, $C\underline{H}_3CH_2$), 2.26-2.47 (2H, m, 3-CH_2), 2.80-2.87 (1H, m, OH), 3.91 (1H, d, *J* 10.8 Hz, 5-H), 4.06-4.16 (2H, m, 5-H and NH), 4.18-4.28 (1H, m), 4.39-4.59 (4H, m, $CH_3C\underline{H}_2$ x2), 4.66-4.73 (1H, s (br), 4-H), 6.11 (1H, t, *J* 7.5 Hz, 2-H); δ_C 24.2 ($C\underline{Me}_3$), 27.2 and 27.3 (CH_3 x2), 36.9 (CH_2), 38.9 ($\underline{C}Me_3$), 56.9 (CH_2), 63.7 (CH_2), 65.5 (CH_2), 65.9 (CH), 70.4 (CH), 148.1 ($C_{quaternary}$), 148.9 ($C_{quaternary}$), 151.4 ($C_{quaternary}$), 177.5 ($C_{quaternary}$).
NOTE: No molecular ion was found for this compound.

2-*tert*-Butyl-1-aza-3-oxabicyclo[3.3.0]octan-4-one (**102**)

(**102**) was prepared following the procedure given by Glaxo Group Research (Greenford) rather than literature procedure,[123] were difficulties were encountered upon isolation of this material.

Into a 250 ml round bottom flask was placed a suspension of (L)-proline (10.12 g, 0.088 mol), trifluoroacetic acid (0.2 ml) and trimethylacetaldehyde (23 ml, 0.019 mol) in dichloromethane (150 ml). The mixture was heated at

reflux under nitrogen in a Soxhlet apparatus, with dried 3Å molecular sieves in the Soxhlet thimble. After heating for 48h with four changes of fresh molecular sieves and with all the proline in solution. The solvent was then removed under reduced pressure to give a red/brown oil, which was immediately suspended in dry hexane (10 ml) and stoppered. The orange solution was then transferred *via* cannular under nitrogen into a dry 100 ml round bottom flask containing 20 ml of dry hexane. The mixture was then allowed to stand at room temperature for 20 min, so that any unreacted (L)-proline precipitated. The liquid was then transferred into a dry 100 ml round bottom flask again *via* cannular under nitrogen and evaporated under reduced pressure to give the bicycle (**102**) (15.23 mg, 95%) as a red/orange oil, which was immediately sealed and stored under nitrogen.

NOTE:Spectroscopic data was not collected due to the high susceptibility of the compound (**102**) to moisture.

(2R)-Methylbenzyl ether-(2S)-pyrrolidinecarboxylic acid (**105**)

To a solution of diisopropylamine (1.57 ml, 0.01 mol) in THF (15 ml) at -78°C was added dropwise n-butyllithium (1.26M in hexane, 8.9 ml, 0.01 mol). The resulting solution was stirred at -78°C for 15 min and then at 0°C for 5 min. The solution was then recooled to -78°C and a solution of bicycle (**102**) (1.37 g, 7.4 mmol) in THF (2 ml) was added dropwise and the resulting orange solution was then stirred at -78°C for 1h. After that time, a solution of benzyl chloromethyl ether[124] (**103**) (1.2 g, 7.6 mmol) in THF (2 ml) was added dropwise. The mixture was then further stirred at -78°C to -30°C for 2h and at room temperature for 20h. On returning, the mixture was diluted with ether (20 ml) and acidified to pH6 with 2M aqueous hydrochloric acid (6 ml). The ether layer was separated and then washed with water (3 x 10 ml), dried ($MgSO_4$) and concentrated *in vacuo* to give a brown oil which was redissolved

in methanol (6 ml) and water (1.5 ml). To this mixture was added silica gel (Merck 9385 1.9 g). The mixture was then stirred at room temperature for 7h. On returning, the silica gel was removed by filtration and washed with methanol (50 ml). The filtrate was evaporated under reduced pressure to give an orange oil which was purified by chromatography using dichloromethane-methanol (9:1 v/v) and then with methanol as eluant, to give the α-alkylated adduct (**105**) (380 mg, 22%) which recrystallised from ethanol as a colourless solid, m.p. 238-249°C (dec.) (Found: C, 66.2; H, 7.35; N, 5.93. $C_{13}H_{17}NO_3$ requires C, 66.36; H, 7.28; N, 5.95%); υ_{max} (nujol mull) 3075, 2480 and 1610 cm^{-1}; δ_H (DMSO-d_6) 1.62-1.88 (3H, m), 2.00-2.08 (1H, m), 2.98-3.09 (1H, m), 3.23-3.40 (1H, m), 3.56 (1H, d, *J* 9.9 Hz, part of AB system), 5.89 (1H, d, *J* 9.9 Hz, part of AB system), 4.50 (2H, s, $PhC\underline{H}_2O$), 7.36 (5H, s, Ph), 8.30-8.80 (2H, s (br), NH and OH); m/z (+FAB) 236 (M^+ + H, 100%), 190 (15), 91 (38); (-FAB) 234 (M^+ - H, 100%).

1-[N-(1-oxo-2,2-dimethylpropyl)]-(2R)-Methylbenzyl ether-(2S)-pyrrolidine-carboxylic acid (**107**)

To an ice-cooled solution of α-benzylmethylether adduct (**105**) (830 mg, 3.5 mmol) in pyridine (3 ml) was added trimethylacetyl chloride (0.46 ml, 3.7 mmol). The mixture was stirred at O°C for 2h and then at room temperature for 20h. After that time, the mixture was diluted with ethyl acetate (30 ml) and washed with 1M aqueous hydrochloric acid (15 ml) and the aqueous layer was then further extracted with ethyl acetate (10 ml). The combined organic extracts were dried (Na_2SO_4) concentrated *in vacuo* and the residue purified by chromatography, using ethyl acetate-petrol as eluant, to afford the N-protected pyrrolidine (**107**). Recrystallisation from ethyl acetate-petrol afforded (**107**) (751 mg, 68%). m.p. 112-113°C $[\alpha]^{21}_D$+56.9° (c 1.06 in methanol)(Found: C, 67.4; H, 7.83; N, 4.33. $C_{18}H_{25}NO_4$ requires C, 67.69; H,

7.89; N, 4.38%); υ_{max} (nujol mull) 3020 (br) and 1725 cm^{-1}; δ_H 1.27 (9H, s, $COCMe_3$), 1.93-2.09 (2H, m), 2.19-2.32 (2H, m), 3.57-3.70 (1H, m), 3.82-3.94 (2H, m), 4.13-4.22 (1H, m), 4.49-4.60 (2H, m, $PhC\underline{H}_2O$), 7.24-7.39 (5H, m, Ph), (1 x OH not seen); m/z (C.I.) 320 (M^+ + H, 100%), 274 (10), 212 (24), 200 (18), 91 (47).

1-[N-(1-oxo-2,2-Dimethylpropyl)]-(2R)-Methylbenzyl ether-N--(phenylmethoxy)-(2S)-pyrrolidinecarboxamide (**108**)

Pyrrolidine (**107**) (319 mg, 0.98 mmol) in dichloromethane (6 ml) was coupled to O-benzylhydroxylamine (124 mg, 1.0 mmol) in the presence of 1-hydroxybenzotriazole hydrate (178 mg, 1.3 mmol) and dicyclohexyl-carbodiimide (274 mg, 1.3 mmol) as described for the conversion of (**62**) to (**80**) and pyrrolidinecarboxamide (**108**) (248 mg, 60%) was isolated as a colourless oil (Found: M^+ + H, 425.2440, $C_{25}H_{33}N_2O_4$ requires 425.2440); υ_{max} (thin film) 3270 (br), 1670 and 1625 cm^{-1}; δ_H 1.22 (9H, s, $COCMe_3$), 1.78-1.99 (2H, m), 2.05-2.21 (2H, m), 3.53 (1H, d, *J* 9.2 Hz, part of AB system), 3.57-3.64 (1H, m), 3.76-3.85 (1H, m), 4.23 (1H, d, *J* 9 Hz, part of AB system), 4.34 (1H, d, *J* 11.7 Hz, part of A′B′ system), 4.42 (1H, d, *J* 11.7 Hz, part of A′B′ system), 4.86 (1H, d, *J* 11.2 Hz, part of A″B″ system), 4.94 (1H, d, *J* 11.4 Hz, part of A″B″ system), 7.12-7.19 (2H, m), 7.26-7.39 (6H, m), 7.41-7.46 (2H, m), 9.57 (1H, s, NH); δ_C 24.3 (CH_2), 27.2 ($C\underline{Me}_3$), 33.2 (CH_2), 39.2 ($\underline{C}Me_3$), 49.8 (CH_2), 70.4 (2-$C_{quaternary}$), 70.8 (CH_2), 77.8 (CH_2), 127.5-129.3 (Ph_{CH}), 135.6 ($Ph_{Cquaternary}$), 137.3 ($Ph_{Cquaternary}$), 170.5 (C=O),178.5 (NC=O); m/z (+FAB) 425 (M^+ + H, 37%), 302 (87), 91 (100); (-FAB) 423 (M^+ - H, 100%), 318 (4).

[N-(1-oxo-2,2-Dimethylpropyl)]-(2R)-Methylbenzyl ether-N-(hydroxy)--(2S)-pyrrolidinecarboxamide (**109**)

To a solution of O-benzylhydroxamic acid (**108**) (62 mg, 0.14 mmol) in methanol (3 ml) was added 10% palladium over charcoal catalyst (17 mg) and the mixture was then subjected to atmospheric hydrogenation for 1.45h. After that time, the catalyst was removed by filtration through celite and the solids washed with methanol (15 ml). The filtrate was evaporated under reduced pressure to give an oil which was purified by chromatography, using ethyl acetate-petrol (1:1 v/v) as eluant, to give hydroxamic acid (**109**) (34 mg, 72%) as a colourless oil (Found: M^+ + H, 335.1971. $C_{18}H_{27}N_2O_4$ requires 335.1970); υ_{max} (thin film) 3250 (br), 1660 and 1615 (br); δ_H 1.24 (9H, s, $COCMe_3$), 1.81-2.05 (2H, m), 2.16-2.28 (2H, m), 3.61 (1H, d, *J* 9 Hz, part of AB system), 3.61-3.69 (1H, m), 3.81-3.90 (1H, m), 4.25 (1H, d, *J* 9 Hz, part of AB system), 4.47 (1H, d, *J* 12 Hz, part of A′B′ system), 4.63 (1H, d, *J* 12 Hz, part of A′B′ system), 7.28-7.42 (5H, m, Ph), 7.79-8.20 (1H, s (br), OH), 9.70 (1H, s, NH); δ_C 24.4 (CH_2), 27.2 ($C\underline{Me}_3$), 33.3 (CH_2), 39.3 ($\underline{C}Me_3$), 49.9 (CH_2), 70.3 (CH_2), 71.4 (2-$C_{quaternary}$), 73.4 (CH_2), 127.6-128.4 (Ph_{CH}), 137.3 ($Ph_{Cquaternary}$), 171.9 (C=O), 177.1 (NC=O); m/z (+FAB) 335 (M^+ + H, 54%),302 (100), 374 (54), 190 (22), 91 (77). (-FAB) 333 (M^+ - H, 100%), 311 (25), 227 (28), 135 (73), 106 (88).

1-[N-(1-oxo-2,2-Dimethyl-1-oxopropy)]-(2R)-Methylbenzyl ether-(2S)--pyrrolinecarboxamide (**110**)

To a solution of N-(hydroxy)-(2S)-pyrrolidinecarboxamide (**109**) (88 mg, 0.26 mmol) in methanol (4 ml) was added ammonium formate (80 mg, 1.2 mmol) and 10% palladium over charcoal catalyst (46 mg). The mixture was stirred at room temperature for 1h and then heated at 70-75°C for 2h. After that time,

the catalyst was removed by filtration through celite and washed with methanol (10 ml). The filtrate was evaporated under reduced pressure to give an oil which was dissolved in ethyl acetate (15 ml) and washed with brine (3 ml). The ethyl acetate extract was dried (Na_2SO_4) and concentrated *in vacuo* to give an oil which was purified by chromatography, using ethyl acetate-petrol (7:3 v/v) as eluant, to afford amide (**110**) (30 mg, 36%) as a colourless solid, m.p. 126-128°C (ethyl acetate-petrol) (Found: $M^+ + H$, 319.2022. $C_{18}H_{27}N_2O_3$ requires 319.2021); υ_{max} (nujol mull) 3460, 3280, 1685 and 1615 cm^{-1}; δ_H 1.25 (9H, s, $COCMe_3$), 1.82-2.02 (2H, m), 2.08-2.24 (2H, m), 3.54 (1H, d, *J* 9 Hz, part of AB system), 3.64 (1H, td, *J* 9.8 and 7 Hz), 3.87 (1H, td, *J* 9.9 and 6 Hz), 4.40 (1H, d, *J* 9 Hz, part of AB system), 4.49 (1H, d, *J* 11.9 Hz, part of A'B' system), 4.63 (1H, d, *J* 11.9 Hz, part of A'B' system), 5.22-5.29 (1H, m, NH), 6.82-6.98 (1H, s (br), NH), 7.30-7.38 (5H, m, Ph); δ_C 24.6 (CH_2), 27.3 ($C\underline{Me}_3$), 33.5 (CH_2), 39.2 ($\underline{C}Me_3$), 49.8 (CH_2), 70.6 (2-$C_{quaternary}$), 71.2 (CH_2), 73.4 (CH_2), 127.6-128.5 (Ph_{CH}), 137.4 ($Ph_{Cquaternary}$), 175.1 (C=O), 177.3 (NC=O); m/z (C.I.) 319 ($M^+ + H$, 11%), 302 (100), 274 (58), 229 (30); (+ FAB) 319 ($M^+ + H$, 100%), 302 (79), 274 (83), 91 (70).

Further attempts to remove the O-benzyl residue in compound (**109**) using boron trifluroetherate and ethanethiol[125] and dissolving metal reduction (Na/EtOH)[126] were unsuccessful.

1-[N-(1-oxo-2,2-dimethylpropyl)]-(2R)-hydroxymethyl-(2S)-pyrrolidine-carboxylic acid (**111**)

Pyrrolidine derivative (**107**) (188 mg, 0.59 mmol) in methanol (7 ml) was O-debenzylated in the presence of 10% palladium over charcoal catalyst (42 mg) under atmospheric hydrogenation conditions. The reaction took 1.45h to

complete. The mixture was filtered through celite and the solids washed with methanol (20 ml). Evaporation of the filtrate under reduced pressure afforded the 2-(hydroxymethyl) derivative (**111**) (133 mg, 98%) as a colourless solid, m.p. 286-288°C (ethanol-ether) (Found: C, 57.4; H, 8.35; N, 5.93. $C_{11}H_{19}NO_4$ requires C, 57.62; H, 8.35; N, 6.10%); υ_{max} (nujol mull) 3400 and 1725 cm^{-1}; δ_H (Methanol-d_4) 1.26 (9H, s, $COCMe_3$), 1.93-2.08 (3H, m), 2.21-2.31 (1H, m), 3.61-3.70 (1H, m), 3.84 (1H, d, *J* 11.7 Hz, part of AB system), 3.94-4.05 (1H, m), 4.13 (1H, d, *J* 11.3 Hz, part of AB system), (2 x OH not seen); m/z (C.I.) 230 (M^+ + H, 100%), 212 (7), 198 (3).

1-[N-(1-oxo-2,2-Dimethylpropyl)]-(2R)-(hydroxymethyl)-N--(phenylmethoxy)-(2S)-pyrrolidinecarboxamide (**112**) and (2R)-[Hydroxymethyl-(1,1-dimethylpropanoate)]-2-(N-phenylmethoxyl)--(2S)-pyrrolidinecarboxamide (**113**)

The 2-(hydroxymethyl) derivative (**111**) (690 mg, 3.0 mmol) was suspended in dichloromethane (10 ml) and dimethylformamide (3 ml) and coupled to O-benzylhydroxylamine (471 mg, 3.8 mmol) in the presence of 1-hydroxy--benzotriazole hydrate (511 mg, 3.7 mmol) and dicyclohexylcarbodiimide (780 mg, 3.7 mmol) as described for the conversion of (**61**) to (**80**). The mixture was stirred at room temperature for 2 days. After that time, the dicyclohexylurea was removed by filtration and washed with ethyl acetate (25 ml). The filtrate was evaporated under reduced pressure to give an oil which was dissolved in ethyl acetate (30 ml), washed with saturated aqueous ammonium chloride solution (5 ml), dried (Na_2SO_4) and concentrated *in vacuo* to give an oil. Purification by chromatography, using ethyl acetate-petrol as eluant, afforded 1,1-dimethylpropanoate derivative (**113**) (85 mg, 8%) followed by the N-(phenylmethoxy)-2-pyrrolidinecarboxamide (**112**) (301 mg, 30%) as colourless crystals.

Compound (**112**) m.p. 156-5-157-5°C (ethyl acetate-petrol) (Found: C, 64.3; H, 7.83; N, 8.31. $C_{18}H_{26}N_2O_4$ requires C, 64.65; H, 7.77; N, 8.36%); υ_{max} (nujol mull) 3240, 1650 and 1620 cm^{-1}; δ_H 1.25 (9H, s, $COCMe_3$), 1.60-1.71 (1H, m), 1.79-1.90 (1H, m), 2.05-2.19 (2H, m), 3.53-3.63 (1H, m), 3.70 (1H, d, *J* 12.5 Hz, part of AB system), 3.87 (1H, d, *J* 12.3 Hz, part of AB system), 3.95-4.03 (1H, m), 4.89 (2H, s, $NOCH_2$), 7.32-7.45 (5H, m, Ph), 7.76-7.81 (1H, m, OH), 9.56 (1H, s, NH); m/z (C.I.) 335 (M^+ + H, 8%), 184 (100), 154 93), 91 (62).

(**113**): isolated as a colourless oil (Found: M^+ + H 335.1971. $C_{18}H_{27}N_2O_4$ requires 335.1970); υ_{max} (thin film) 3280, 3140 (br), 1725 and 1655 (br) cm^{-1}; δ_H 1.18 (9H, s, $COCMe_3$), 1.72-1.83 (3H, m), 1.85-2.02 (1H s (br), NH), 2.04-2.18 (1H, m), 2.76-2.85 (1H, m), 2.90-3.01 (1H, m), 4.12 (1H, d, *J* 11.2 Hz, part of AB system), 4.42 (1H, d, *J* 11.2 Hz, part of AB system), 4.87 (1H, d, *J* 11.2 Hz, part of A′B′ system), 4.98 (1H, d, *J* 11 Hz, part of A′B′ system), 7.34-7.44 (5H, m, Ph), 9.82-10.02 (1H, s (br), NH); δ_C 25.0 (CH_2), 27.0 ($C\underline{Me}_3$), 33.4 (CH_2), 38.7 ($C_{quaternary}$), 46.7 (CH_2), 66.9 (CH_2), 69.0 ($C_{quaternary}$), 78.0 (CH_2), 128.4-129.7 (Ph_{CH}), 135.0 ($Ph_{Cquaternary}$), 170.7 (C=O), 178.1 (NC=O); m/z (C.I.) 335 (M^+ + H, 10%), 184 (100), 91 (65).

Further attempts to increase the yield of compound (**112**) such as changing reaction conditions and using more reactive coupling agents, such as BOP-Cl and BOP Reagent were unsuccessful (*See Chapter 4*; *Table 8).*

1-[N-(1-oxo-2,2-dimethylpropyl)]-(2R)-(Hydroxymethyl)-N-(hydroxy)-(2S)--pyrrolidinecarboxamide (**114**)

Pyrrolidine (**112**) (99 mg, 0.29 mmol) in methanol (5 ml) was debenzylated in the presence of 10% palladium over charcoal catalyst (23 mg) under atmospheric hydrogenation for 3.5h. The catalyst was removed by filtration

through celite and the solids washed with methanol (15 ml). The filtrate was evaporated under reduced pressure to give a solid. Recrystallisation from ethanol-ether afforded hydroxamic acid (**114**) (46mg, 65%) as a colourless needles m.p. 153.5-155.8°C (Found: C, 54.0; H, 8.34; N, 11.47. $C_{11}H_{20}N_2O_4$ requires C, 54.08; H, 8.25; N, 11.46%); υ_{max} (nujol mull) 3440, 3250, 3150, 1620 cm^{-1}; δ_H (Methanol-d_4) 1.25 (9H, s, $COCMe_3$), 1.94-2.05 (3H, m), 2.19-2.25 (1H, m), 3.70 (1H, td, *J* 9.9 and 6.8 Hz), 3.85 (1H, d, *J* 11 Hz, part of AB system), 3.87-3.97 (1H, m), 4.05 (1H, d, *J* 11 Hz, part of AB system), 4.89 (2H, s, NH and OH / 2 x OH), (1 x OH / NH not seen); m/z (+FAB) 245 (M^+ + H, 50%), 212 (100), 184 (60); (-FAB) 243 (M^+ - H, 100%), 182 (7), 153 (17).

4-[(N-1-oxo-2,2-dimethylpropyl)3-aminopropyl]-4-isoxazoline-3-one (**115**)

To an ice-cooled solution of triphenylphosphine (47 mg, 0.18 mmol) and diethylazodicarboxylate (0.028 ml, 0.18 mmol) in THF (2 ml) was added hydroxamic acid (**114**) (26 mg, 0.1 mmol). The mixture was stirred at 0°C for 2.5h and then at room temperature for 20h. On returning, the solvent was evaporated under reduced pressure to give an oil which was purified by chromatography, using dichloromethane-methanol (9:1 v/v) as eluant, to give isoxazoline-3-one (**115**) (13.5 mg, 55%) as a colourless oil (Found: M^+ + H, 227.1396. $C_{11}H_{19}N_2O_3$ requires 227.1396); υ_{max} ($CHCl_3$) 3370 (br), 3190 (br), 3020, 1740, 1630 cm^{-1}; δ_H 1.20 (9H, s, $COCMe_3$), 1.71-1.81 (2H, m), 2.34 (2H, t, *J* 6.8 Hz), 3.34 (2H, q, *J* 6.5 Hz), 6.15-6.22 (1H, m, NH), 6.55 (1H, d, *J* 1.3 Hz), 10.18 (1H, s (br), NH); δ_C 20.3 (CH_2), 27.6 ($C\underline{M}e_3$), 29.3 (CH_2), 38.1 (CH_2), 38.8 ($\underline{C}Me_3$), 124.1 (CH), 126.8 ($C_{quaternary}$), 157.0 (C=O), 179.8 (C=O); m/z (C.I.) 227 (M^+ + H, 100%), 169 (12.8), 85 (13).

NOTE: An alternative method for cyclisation of hydroxamic acid (**114**) to the *spiro*-bicycle (**IV**) using $PPh_3/CCBr_4$ was unsuccessful.

N-Chloromethoxyphthlalimide (**118**)[134]

To a suspension of N-hydroxyphthalimide (104 mg 0.64 mmol) in bromochloromethane (4 ml) was added anhydrous silver(I) oxide (182 mg, 0.78 mmol). The suspension was heated at 75-80°C for 1h. On returning, the cooled mixture was filtered through celite and washed with methanol (10 ml). The filtrate was evaporated under reduced pressure to give N-chloromethoxyphthalimide (**118**) as a colourless solid (125 mg, 92%). Recrystallisation from ethyl acetate-petrol afford (**118**) (80 mg, 58%) as colourless needles, m.p. 129-130°C (lit.,[134] 126-128°C (from $CHCl_3$-petrol)); υ_{max} (nujol mull) 1780 and 1730 cm^{-1}; δ_H 5.88 (2H, s, CH_2O), 7.79-7.83 (2H, m), 7.86-7.90 (2H, m); m/z (70eV E.I.) 211 (M^+, 3%), 176 (15), 147 (27), 105 (100). (C.I.) 212 ($M^+ + H$, 100%), 214 (33), 176 (69), 148 (73), 105 (20).

Reaction of N-chloromethoxyphthalimide (**118**) with diethyl malonate (**119**)

To a cooled solution of diethyl malonate (329 mg, 2.0 mmol) in absolute ethanol (2 ml) was added sodium (52 mg, 2.3 mmol), followed by dropwise addition of a solution of N-chloromethoxyphthalimide (**118**) (226 mg, 1.0 mmol) in absolute ethanol (2 ml). The mixture was then stirred at -15°C for 24h. After that time the mixture was quenched with saturated ammonium chloride solution (5 ml) and extracted with ethyl acetate (4 x 15 ml). The combined ethereal layers were dried (Na_2SO_4) and concentrated *in vacuo* to give an oil. Purification by chromatography on silica gel using ethyl acetate-petrol as eluant, afforded N-ethyloxymethoxyphthalimide (**121**) (59 mg, 25%) and 2-(ethoxymethoxyaminocarbonyl)benzoic acid, ethyl ester (**122**) (28 mg, 27%) as solids.

(**121**) was isolated as a colourless solid, m.p. 75-78°C(ether-petrol); υ_{max}

(nujol mull) 1720 cm^{-1} and 1450 cm^{-1}; δ_H (60 MHz) 1.25 (3H, t, *J* 7 Hz, $CH_2\underline{CH_3}$), 4.00 (2H, q, *J* 7 Hz, $\underline{CH_2}CH_3$), 5.10 (2H, s, $O\underline{CH_2}O$), 7.70 (5H, s, Ph); m/z (C.I.) 222 ($M^+ + H$, 100%), 176 (28), 163 (37), 148 (73).

NOTE: Unsatisfactory microanalytical data was obtained for the above compound (**121**).

(**122**): Isolated as a colourless solid, m.p. 58-59°C (ether-petrol) (Found: C, 58.3; H, 6.38; N, 5.15. $C_{13}H_{17}NO_5$ requires C, 58.41; H, 6.41; N, 5.24%); υ_{max} (nujol mull) 3140, 1710 and 1630 cm^{-1}; δ_H (60 MHz) 1.05-1.45 (6H, m, $CH_2\underline{CH_3}$ x2), 3.55-3.95 (4H, m, $\underline{CH_2}CH_3$ x2), 4.95 (2H, s, OCH_2O), 7.45 (4H, s, Ph), 9.15 (1H, s (br), NH); m/z (C.I.) 268 ($M^+ + H$, 4%), 177 (100).

NOTE: Compound (**121**) (19 mg, 16%) and (**122**) (56 mg, 40%) were similarly obtained by adding N-chloromethoxyphthalimide (**118**) (110 mg, 0.5 mmol) in absolute ethanol (2 ml) to an ice-cooled solution of sodium ethoxide [from sodium (12 mg, 0.52 mmol)] in absolute ethanol (3 ml) and stirring the mixture at room temperature for 3h. The structures of (**121**) and (**122**) were confirmed by ^1H NMR and melting point with the samples obtained from the above experiment.

N-Phenylthiomethoxyphthalimide (**125**)

To a suspension of potassium bicarbonate (11.1 g, 0.08 mol) in dichloromethane (20 ml) was added thiophenol (9.1 g, 0.08 mol). The mixture was stirred at room temperature for 1h. After that time, a solution of N-chloromethoxyphthalimide (4.1 g, 0.02 mol) in dichloromethane (10 ml) was added. The resulting mixture was then stirred at 45°C for 3.5 days. On returning, the mixture was washed with saturated ammonium chloride solution (20 ml) and extracted with dichloromethane (3 x 25 ml). The combined dichloromethane extracts were dried (Na_2SO_4) and evaporated under reduced

pressure to give a colourless solid. Purification by chromatography using ethyl acetate-petrol as eluant, afforded a solid which was recrystallised from ethyl acetate-petrol to give (**125**) (4.4 g, 76%) as colourless needles, m.p. 85-86°C (Found: C, 63.4; H, 3.8; N, 4.92. $C_{15}H_{11}NO_3S$ requires C, 63.15; H, 3.89; N, 4.91%); υ_{max} ($CHCl_3$) 3020, 1775, 1710 and 1570 cm^{-1}; δ_H (60 MHz) 5.61 (2H, s, CH_2O), 7.30-7.50 (2H, m), 7.65-7.90 (7H, m); m/z (70eV E.I.) 285 (M^+, 3%), 147 (2). (C.I.) 286 ($M^+ + H$, 3%), 176 (5), 163 (3), 148 (27).

N-Methoxymethyphtalimide (**126**)

To an ice-cooled solution of potassium bicarbonate (53 mg, 0.94 mmol) in methanol (1.5 ml) was added dropwise thiophenol (72 mg, 0.65 mmol). The mixture was stirred at 0°C for 10 min. After that time a solution of N-chloromethoxyphthalimide (106 mg, 0.50 mmol) in methanol (2 ml) was added. The resulting mixture was stirred at 0°C for 6h and then at room temperature for 1.5 days. On returning, the solvent was evaporated under reduced pressure to give an oil. The oil was redissolved in ethyl acetate (10 ml) and washed with saturated ammonium chloride solution (2 ml). The organic extract was dried (Na_2SO_4) and evaporated under reduced pressure to give a solid which was purified by chromatography, using ethyl acetate-petrol as eluant, to give (**126**) (64 mg, 62%) as a colourless solid, m.p. 116-118°C (ethyl acetate-petrol) (Found: C, 57.9; H, 4.3; N, 6.53. $C_{10}H_9NO_4$ requires C, 57.97; H, 4.38; N, 6.76%); υ_{max} (nujol mull) 1700 cm^{-1}; δ_H (60 MHz) 3.07 (3H, s), 5.05 (2H, s), 7.80 (4H, s); m/z (C.I.) 208 ($M^+ + H$, 100%), 176 (38), 163 (23), 148 (81).

Benzylidene glycine ethyl ester (**127**) was prepared following literature procedures.[140]

Reaction between N-Chloromethoxyphthalimide and Imine (**127**)

To an ice-cooled suspension of potassium *tert*-butoxide (127 mg, 1.1 mmol) in THF (2 ml) at -78°C was added dropwise a solution of imine (**127**) (213 mg, 1.1 mmol) in THF (2 ml) and the mixture was stirred at -78°C for 25 min. After that time, a solution of N-chloromethoxyphthalimide (197 mg, 0.9 mmol) in THF (2 ml) was added to the mixture; the solution turned from red to yellow during the addition. The mixture was stirred at -78°C for 35 min before being quenched with saturated aqueous ammonium chloride solution (3 ml). The mixture was then extracted with ethyl acetate (3 x 6 ml). The combined organic layers were dried (Na_2SO_4) and concentrated *in vacuo* to give a brown oil. This residue was redissolved in absolute ethanol (2 ml), cooled to 0°C and a suspension of sodium borohydride (38 mg, 1.1 mmol) in absolute ethanol (2 ml) was added. The mixture was stirred for 20 min at 0°C before being quenched with 2M aqueous hydrochloric acid (2 ml). The mixture was then neutralised to pH6 with 1M aqueous sodium hydrogen carbonate and extracted with ethyl acetate (3 x 5 ml). The organic layers were dried (Na_2SO_4) and evaporated under reduced pressure to give N-benzylglycine ethyl ester as a brown oil (200 mg, 94%); υ_{max} (thin film) 3300 (br), 2980 and 1720 cm^{-1}; δ_H (60 MHz) 1.33 (3H, t, $CH_2\underline{CH_3}$), 2.34 (1H, s, NH), 3.52 (2H, s), 3.98 (2H, s), 4.37 (2H, q, $C\underline{H_2}CH_3$), 6.80 (5H, s, Ph); m/z (C.I.) 194 ($M^+ + H$, 100%), 148 (45), 120 (33), 91 (90).

Synthesis of N-Iodomethoxyphthalimide(**129**)

To a suspension of N-chloromethoxyphthalimide (1.27 g, 6.0 mmol) in acetone (50 ml) was added anhydrous sodium iodide (10.5 g, 0.07 mol). The mixture was heated at reflux for 24h. After that time, the solvent was evaporated under reduced pressure to give a solid (998 mg). The solid was

suspended in dichloromethane (30 ml) and washed with 5M aqueous sodium thiosulphate (10 ml). The aqueous layer was then further extracted with dichloromethane (3 x 25 ml). The combined organic extracts were dried (Na_2SO_4), filtered and concentrated *in vacuo* to give a solid. Purification by chromatography on silica gel using ethyl acetate-petrol as eluant afforded (**129**) as a solid. Recrystallisation from ethyl acetate-petrol gave (**129**) (709 mg, 39%) as colourless needles, m.p. 115-118°C (Found: C, 35.85; H, 1.94; N, 4.61. $C_9H_6INO_3$ requires C, 35.64; H, 1.98; N, 4.62%); υ_{max} (nujol mull) 1710 cm^{-1}; δ_H (60 MHz) 6.00 (2H, s, $C\underline{H}_2O$), 7.65 (4H, s, Ph); m/z (C.I.) 304 (M^+ + H, 45%), 176 (100), 148 (47).

2-{[3-t-Butyl-5,6,7,7a-tetrahydro-1-oxo-1H,3H-pyrrolo[1,2-c]oxazol-7a-yl]--carbonyl}-N-(chloromethyoxy)benzamide (**130**)

To a solution of diisopropylamine (0.67 ml, 4.7 mmol) in THF (5 ml) at -78°C was added dropwise n-butyllithium (2.5M in hexane, 1.9 ml, 4.7 mmol). The resulting solution was stirred at -78°C for 20 min and then at room temperature for 5 min and recooled back to -78°C before a solution of bicycle acetal (**102**) (0.7 g, 3.8 mmol) in THF (2 ml) was added dropwise. The orange solution was then stirred at -78°C for 30 min. After that time, a solution of N-chloromethoxyphthalimide (118) (0.85 g, 4.0 mmol) in THF (5 ml) was added dropwise. The mixture was then stirred at -78°C to -30°C for 2.5h. On returning, the mixture was diluted with ether (30 ml) and acidified to pH6 with 1M aqueous hydrochloric acid. The aqueous extract was washed with ether (2 x 10 ml). The combine ethereal extracts were dried (Na_2SO_4), filtered and evaporated under reduced pressure to give an orange oil (2 g). Purification by chromatography on silica gel using ethyl acetate-petrol as eluant afforded starting material N-chloromethoxyphthalimide (**118**) (0.3 g, 35%) and then the ring opened phthalimide-adduct (**130**) (24 mg, 2%) as a solid.

Recrystallisation from ethyl acetate-petrol afforded (**130**) as a pale straw crystalline solid, m.p. 157-159°C (dec.) (Found: C, 58.4; H, 6.15; N, 6.98. $C_{19}H_{23}N_2O_5Cl$ requires C, 57.79; H, 5.87; N, 7.98%); υ_{max} (nujol mull) 3300, 1785, 1730 and 1715 cm^{-1}; δ_H 0.92 (9H,s, COC<u>Me</u>$_3$), 1.70-1.89 (2H, m), 2.20-2.37 (2H, m), 2.52-2.61 (1H, m), 2.74-2.81 (2H, m), 3.88 (1H, s(br), NH), 4.10-4.14 (2H, m, CH_2O), 7.50-7.90 (4H, m); m/z (+FAB) 395 (M^+ + H, 36%), 397 (12.8), 359 (6), 315 (4), 182 (100). (-FAB) 393 (M^+ - H, 100%), 395 (40), 312 (13), 188 (48).

<u>N,N'-(Bis-*tert*-butyloxycarbonyl)-O-benzylhydroxylamine (**132**)</u>

To a stirred solution of O-benzylhydroxylamine (330 mg, 2.6 mmol) in dichloromethane (8 ml) was added 4-dimethylaminopyridine (39 mg, 0.32 mmol) followed by dropwise addition of a solution of di-*tert*-butyldicarbonate (1.36 g, 6 mmol) in dichloromethane (8 ml). The yellow solution was then stirred at room temperature for 4h. After that time, another 1.4 equiv. of di-*tert*-butyldicarbonate (810 mg, 3.7 mmol) in dichloromethane (5 ml) and 0.7 equiv. of 4-dimethylaminopyridine (22 mg, 0.18 mmol) was added and the mixture was left to stir at room temperature for 20h. On returning, the solvent was removed under reduced pressure to give an oil which was purified by chromatography, using ethyl acetate-petrol (2:98 v/v to 5:95 v/v) as eluant, to give the N,N'-(bis-*tert*-butyloxycarbonyl) derivative (**132**) (712 mg, 81%) as a colourless solid and further elution afforded the mono-*tert*-butyloxycarbonyl derivative (**131**) (111 mg, 18%) as a colourless oil.

(**132**): Isolated as a crystalline solid, m.p. 83-84°C (ethyl acetate-petrol) (Found: C, 63.0; H, 7.93; N, 4.25. $C_{17}H_{25}NO_5$ requires C, 63.14; H, 7.79 N, 4.33%); υ_{max} (thin film) 2975, 2935 and 1740 cm^{-1}; δ_H 1.54 (18H, s, COC<u>Me</u>$_3$ x 2), 4.90 (2H, s, O<u>CH</u>$_2$), 7.34-7.46 (5H, m, Ph); m/z (+FAB) 324 (M^+ + H, 8%), 91 (100).

(**131**): Isolated as a colourless oil; υ_{max} (thin film) 2930, 1740 (br) cm^{-1}; δ_H 1.50 (9H, s, $COCMe_3$), 4.19 (2H, s, OCH_2), 7.32-7.47 (5H, m, Ph); δ_C 27.9 ($C\underline{Me}_3$), 78.9 (CH_2) 84.6 ($\underline{C}Me_3$), 128.4-129.7 (Ph_{CH}), 134.4 ($Ph_{Cquaternary}$), 150.6 (C=O); m/z (70eV E.I.) 223 (M^+, 0.8%), 167 (4.6), 91 (75), 57 (100). This compound was not characterised further as it was of no major importance to us.

N,N'-(Bis-*tert*-Butyloxycarbonyl)hydroxylamine (**133**)

O-Benzylhydroxamte (**132**) (632 mg, 1.9 mmol) in methanol (25 ml) was debenzylated in the presence of 10% palladium over charcoal catalyst (128 mg) under atmospheric hydrogenation for 6.5h. After that time, the catalyst was removed by filtration through celite and the solids washed with methanol (40 ml). The filtrate was evaporated under reduced pressure to give a solid. Recrystallisation from ethyl acetate-petrol gave (**133**) (346 mg, 78%) as a colourless crystalline solid, m.p. 88.5-89.5°C (Found: C, 51.6; H, 8.39; N, 5.89. $C_{10}H_{19}NO_5$ requires C, 51.49; H, 8.21; N, 6.00%); υ_{max} (nujol mull) 3300 and 1755 cm^{-1}; δ_H 1.54 (18H, s, $COCMe_3$ x2), 6.80 (1H, s(br), OH); δ_C 28.0 ($C\underline{Me}_3$), 84.6 ($\underline{C}Me_3$), 151.0 (C=O); m/z (+FAB) 234 (M^+ + H, 37%), 178 (100); (-FAB) 232 (M^+ - H, 100%), 216 (46), 131 (17).

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APPENDIX

X-Ray Data for 1-[N-(Hydroxyimino)-2-oxa-5-azabicyclo[2.2.1]heptane (95)

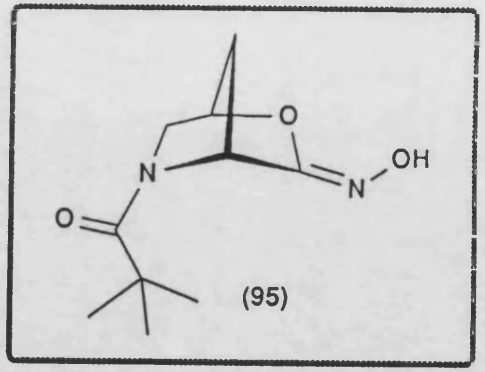
Azabicyclo (**95**) (Figure 27) was recrystallised from ethanol-ether in space group $P2_12_12_1$, a = 5.669(2), b = 11.570(3), c = 15.435(3)Å, U = 1071.8Å^3 and D_c = 1.32 gcm^{-3} for Z = 4 at room temperature. The data were measured on a Hilger and Watts Y290 four-circle diffractometer in the range of $2 \leq \theta \leq 24^\circ$ and were corrected for Lorentz and polarization effects but not for absorption. The structure was resolved by Direct methods using 1015 reflections of which 721 were unique with $I \geq 3\sigma(I)$ and refined using SHELX[141a,b] suite of programs. In the final least squares cycles, the oxygen atoms and N1 were allowed to vibrate anisotropically. All other atoms were treated isotropically. Hydrogen atoms were included at calculated positions. Final residuals after 10 cycles of least squares were R = 0.0894, R_w = 0.0949. Maximum final shift/esd was 0.001. The maximum and minimum residual densities were 0.16 and -0.18eÅ^{-3} respectively. Final fractional atomic coordinates and isotropic thermal parameters, bond distances and angles are given in Tables 12, 13, 14, 15, 16, 17 and 18 respectively.

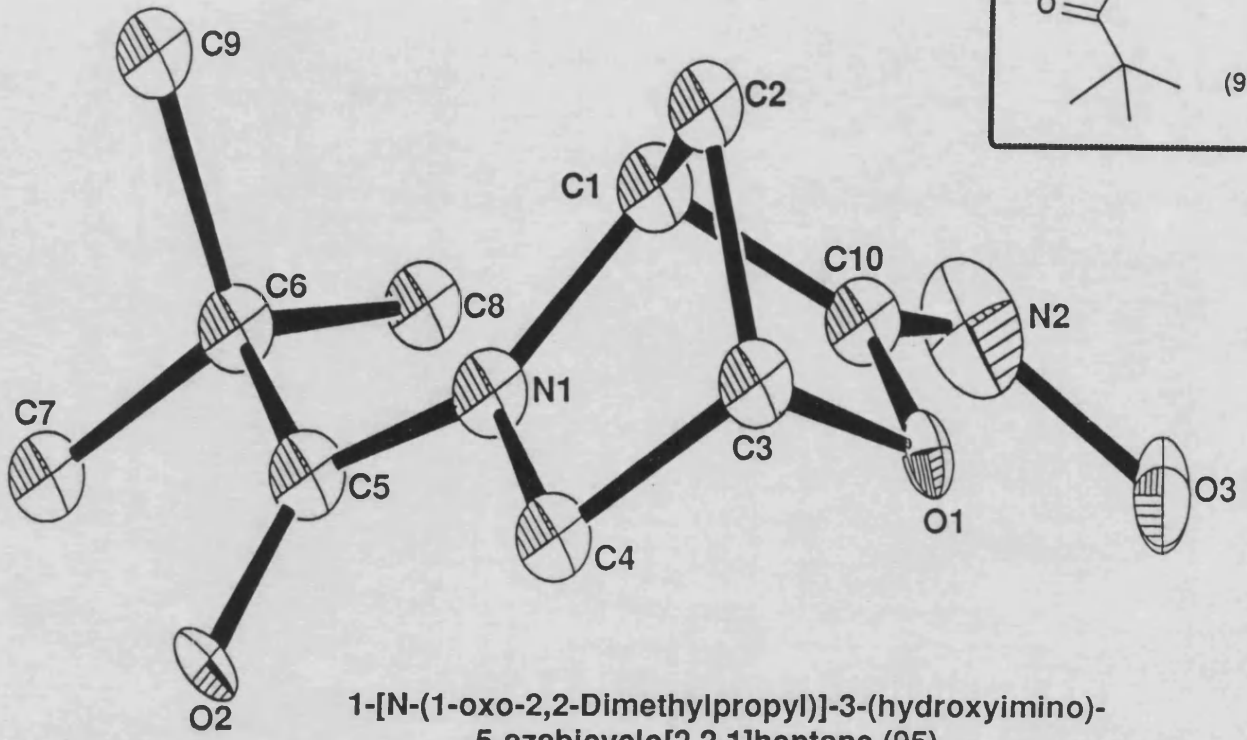
Crystal Data

A crystal of approximate dimensions 0.3 x 0.3 x 0.3 mm was used for data collection.

Molecular Formular $C_{10}H_{16}O_3N_2$, M = 212.3, orthorhombic.

Fig.27





1-[N-(1-oxo-2,2-Dimethylpropyl)]-3-(hydroxyimino)-
-5-azabicyclo[2.2.1]heptane (95)

Table 12

Fractional Atomic Coordinates and Thermal Parameters (Å)

Atom	x	y	z	Uiso or Ueq	(***)
N2	-0.1573(18)	0.4750(7)	0.2218(5)	0.079(6)	***
O1	-0.2176(13)	0.2981(4)	0.2771(3)	0.026(4)	***
O2	0.0285(12)	0.4210(5)	0.5424(3)	0.026(4)	***
O3	-0.3935(15)	0.4587(7)	0.1902(5)	0.047(5)	***
N1	0.0745(14)	0.3747(5)	0.4044(4)	0.060(2)	
C1	0.1366(18)	0.3760(7)	0.3122(5)	0.040(2)	
C2	0.1729(21)	0.2562(8)	0.2930(7)	0.054(3)	
C3	-0.0642(19)	0.2286(7)	0.3285(5)	0.043(2)	
C4	-0.0631(19)	0.2746(7)	0.4191(5)	0.044(2)	
C5	0.1129(17)	0.4458(7)	0.4723(5)	0.036(2)	
C6	0.2592(16)	0.5481(7)	0.4592(5)	0.039(2)	
C7	0.2513(23)	0.6179(10)	0.5420(7)	0.066(3)	
C8	0.1660(22)	0.6219(8)	0.3858(6)	0.053(3)	
C9	0.5142(26)	0.5188(11)	0.4399(8)	0.076(4)	
C10	-0.0888(18)	0.3902(7)	0.2654(5)	0.041(2)	

Table 13

Fractional Atomic Coordinates for the Hydrogen Atoms

Atom	x	y	z
H031	-0.4495	0.5298	0.1543
H032	-0.5114	0.4456	0.2441
H033	-0.3966	0.3881	0.1482
H11	0.2750	0.4336	0.2973
H21	0.3172	0.2201	0.3286
H22	0.1924	0.2382	0.2249
H31	-0.1096	0.1430	0.3276
H41	0.0211	0.2204	0.4648
H42	-0.2398	0.2928	0.4410
H71	0.3148	0.5705	0.5962
H72	0.0720	0.6433	0.5543
H73	0.3616	0.6890	0.5336
H81	0.1671	0.5770	0.3255
H82	0.2777	0.6929	0.3798
H83	-0.0120	0.6473	0.4005
H101	0.5225	0.4695	0.3819
H102	0.5862	0.4733	0.4937
H103	0.6153	0.5925	0.4305

Table 17

Intermolecular Distances (Å)

N2	...H71	2.80	2	0.0	1.0	0.0
N2	...H31	2.66	3	0.0	-1.0	0.0
O1	...H21	2.91	1	1.0	0.0	0.0
O1	...H82	2.77	3	0.0	0.0	0.0
O2	...H102	2.69	1	1.0	0.0	0.0
O2	...O3	2.82	2	-1.0	1.0	-1.0
O2	...H031	1.88	2	-1.0	1.0	-1.0
O2	...H033	2.95	2	-1.0	1.0	-1.0
O2	...H21	2.90	4	0.0	0.0	1.0
O2	...H31	2.98	4	-1.0	0.0	1.0
O2	...H42	2.94	4	-1.0	0.0	1.0
O3	...H11	2.52	1	1.0	0.0	0.0
O3	...H71	2.82	2	-1.0	1.0	0.0
O3	...H72	2.64	2	-1.0	1.0	0.0
H031	...C5	2.97	2	-1.0	1.0	0.0
H032	...C1	2.41	1	1.0	0.0	0.0
H033	...C7	2.60	2	-1.0	1.0	0.0
C4	...H41	2.96	4	0.0	0.0	1.0
C4	...H42	2.95	4	-1.0	0.0	1.0

Table 18

Intramolecular Distances (Å)

N1	...O1	2.73	N1	...O2	2.22
N1	...H11	2.13	N1	...C2	2.32
N1	...H21	2.62	N1	...C3	2.28
N1	...H41	2.13	N1	...H42	2.12
N1	...C6	2.52	N1	...H81	2.81
N1	...H101	2.81	N1	...C10	2.34
N2	...O1	2.35	N2	...H031	2.07
N2	...H032	2.07	N2	...H033	2.06
N2	...C1	2.49	N2	...H11	2.76
N2	...H81	2.74	O1	...O3	2.58
O1	...H032	2.51	O1	...H033	2.49
O1	...C1	2.29	O1	...C2	2.29
O1	...H22	2.57	O1	...H31	2.14
O1	...C4	2.38	O1	...H42	2.53
O2	...C4	2.67	O2	...H41	2.73
O2	...H42	2.69	O2	...C6	2.40
O2	...C7	2.72	O2	...H71	2.58
O2	...H72	2.74	O3	...C10	2.24
H032	...C10	2.51	H033	...C10	2.51
C1	...H21	2.18	C1	...H22	2.18
C1	...C3	2.15	C1	...C4	2.36
C1	...C5	2.62	C1	...H81	2.48
C1	...H101	2.69	H11	...C2	2.25
H11	...C5	2.86	H11	...C6	2.87
H11	...C8	2.75	H11	...C9	2.79
H11	...C10	2.19	C2	...H31	2.18
C2	...C4	2.37	C2	...H41	2.82
C2	...C10	2.25	H21	...C3	2.16
H21	...C4	2.65	H22	...C3	2.16
H22	...C10	2.53	C3	...H41	2.16
C3	...H42	2.15	C3	...C10	2.21
H31	...C4	2.16	C4	...C5	2.46
C4	...C10	2.77	H41	...C5	2.81
H42	...C5	2.78	C5	...C7	2.49
C5	...H71	2.70	C5	...H72	2.74
C5	...C8	2.55	C5	...H81	2.79
C5	...H83	2.80	C5	...C9	2.49
C5	...H101	2.72	C5	...H102	2.72
C6	...H71	2.15	C6	...H72	2.15
C6	...H73	2.15	C6	...H81	2.16
C6	...H82	2.16	C6	...H83	2.16
C6	...H101	2.14	C6	...H102	2.14
C6	...H103	2.14	C7	...C8	2.46
C7	...H82	2.67	C7	...H83	2.67
C7	...C9	2.48	C7	...H102	2.70
C7	...H103	2.70	H71	...C9	2.74
H72	...C8	2.67	H73	...C8	2.67
H73	...C9	2.68	C8	...C9	2.49
C8	...H101	2.75	C8	...H103	2.66
H81	...C9	2.74	H81	...C10	2.86
H82	...C9	2.68			